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Phylogenetic relationships of the genus *Phanerochaete* inferred from the internal transcribed spacer region

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Phanerochaete is a genus of resupinate homobasidiomycetes that are saprophytic on woody debris and logs. Morphological studies in the past indicated that *Phanerochaete* is a heterogeneous assemblage of species. In this study the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA was used to test the monophyly of the genus *Phanerochaete* and to infer phylogenetic relationships of the 24 taxa studied. Maximum parsimony, maximum likelihood, and Bayesian analyses do not support the monophyly of the genus. However, a core group of species represented by *Phanerochaete velutina, P. chrysosporium, P. sordida, P. sanguinea* and others are closely related and group together in a clade. Other common *Phanerochaete* species including *Phanerochaete rimosa, P. chrysorhiza, P. omnivora, P. avellanea, P. tuberculata, P. flava,* and *P. allantospora,* however, do not cluster with the core *Phanerochaete* group.

INTRODUCTION

Phanerochaete P. Karsten (Karsten 1889; *Polyporales, Phanerochaetaceae* fide Kirk *et al.* 2001) is a genus of saprophytic homobasidiomycetes on woody debris and logs. Due to its ability to degrade lignin selectively, one species, *viz. Phanerochaete chrysosporium,* is widely used as a model white-rot fungus (Cullen & Kersten 1996) and may have potential use in industrial applications such as biopulping (Akhtar *et al.* 1998).

Phanerochaete is a morphologically heterogeneous genus characterized by resupinate, effused fruit bodies with smooth, tuberculate to spinose hymenial surfaces, a monomitic hyphal system with primarily simple-septate generative hyphae, clavate basidia, and inamyloid, acyanophilous, thin-walled basidiospores (Eriksson, Hjortstam & Ryvasden 1978, Wu 1995, 1998). Currently, more than 90 species of *Phanerochaete* are described worldwide (Parmasto 1997, Cortbase ver. 1.4, 2002) and additional new species are anticipated (e.g. de Koker, Burdsall & Janse 2000).

In a monograph of *Phanerochaete,* Burdsall (1985) recognized 46 species distributed in three subgenera. The subgenera were distinguished on the presence of cystidia (subgen. *Phanerochaete*), if cystidia arose from the substrate or subiculum, sometimes called pseudocystidia (subgen. *Scopuloides*), or if cystidia and pseudocystidia were absent (subgen. *Phanericium*). Burdsall (1985) relied heavily upon morphology to postulate phylogenetic relationships and to define the subgenera. Thus, the subgenera, as used in that work, are meaningful only in grouping species with similar morphology with respect to cystidia. This makes the subgenera designations similar to form genera used in conidial fungi, useful for the identification of species, but of questionable value in identifying relationships.

The ITS region has been used extensively to infer phylogenetic relationships among wood decay basidiomycetes species within a particular genus or even within a species complex (e.g. Gottlieb, Wright & Moncalvo 2002, Johannesson, Renvall & Stenlid 2000, Lim 2001, Paulus *et al.* 2000). However, Taylor, Bruns & White (1990) showed that variability in a given region varies for different fungal taxa; thus, no single region can be used to infer phylogenetic relationships for all fungi. Species of *Phanerochaete* have been included in several studies that employed the ITS region to infer phylogenetic relationships. Boidin, Mugnier & Canales (1998) included five species of *Phanerochaete* in a large phylogenetic study of the *Aphyllophorales.* In their analysis, *P. sordida, P. chrysosporium, P. velutina, P. burtii* and *P. affinis* formed a monophyletic clade

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that also included *Phlebia subserialis* and *Cotylidia diaphana*. In another phylogenetic study using the ITS region, Lim (2001) included 11 species of *Phanerochaete* as well as several unidentified isolates. In that study, all the species of *Phanerochaete* clustered in a monophyletic clade except for *P. chrysorhiza* that was placed in a separate clade with *Tyromyces alborubescens* and *Phlebia tremellosa*.

We applied sequence data from the ITS region to infer the phylogeny of selected species of *Phanerochaete* and to determine if the genus is monophyletic. We amplified and sequenced ITS fragments from 38 isolates representing 24 species of *Phanerochaete*. The sequences were aligned and analysed using parsimony, maximum likelihood and Bayesian methods.

MATERIALS AND METHODS

Taxon selection

Table 1 lists the species and GenBank accession nos. for ITS sequences used in this study. 24 taxa of *Phanerochaete* are included in this study, and authenticated cultures were selected to represent the three subgenera recognized by Burdsall (1985). Hibbett & Donoghue (2001) showed that *Phanerochaete* is a part of the *Phlebia* subclade that resides in the larger polyporoid clade. Based on this study and previous molecular studies of the ITS region (Boidin *et al.* 1998, Yao, Pegler & Chase 1999, Lim 2001), 15 species allied to *Phanerochaete* were included. *Antrodia xantha*, *Tyromyces alborubescens*, *Ceriporiopsis* spp., and *Diplomitoporus lindbladii* were chosen as outgroup taxa.

Cultures of *Phanerochaete* and related species were obtained from the Center for Forest Mycology Research (CFMR), Forest Products Laboratory, Madison, WI. The South African strain of '*P. pseudomagnoliae*' (nom. prov.) was isolated as described earlier (de Koker *et al.* 2000) and is on deposit at CFMR.

DNA isolation, ITS amplification and sequencing

Genomic DNA was isolated as described by Raeder & Broda (1985). The ITS region was amplified using the ITS4 and ITS5 primers as described by White *et al.* (1990). Expand™ High Fidelity DNA Polymerase (Boehringer Mannheim) was used for PCR reactions using the cycling parameters as described by White *et al.* (1990) on a PerkinElmer GeneAmp 2400 PCR system. Products were purified through Sephadex G-50 (Sigma, St Louis) columns and both strands sequenced using the Big Dye DNA Sequencing Kit (Applied Biosystems, Foster City, CA) with reactions run on a PerkinElmer AB1 PRISM™ 377 DNA sequencer. Primers ITS1 and ITS4 were used for the forward and reverse reactions, respectively. The data from the forward and reverse sequences were analysed and aligned using PC/GENE 6.6 (IntelliGenetics,

Table 1. List of species, strain, and accession numbers for ITS sequences used in the analysis.

Species	Strain[a]	NCBI[b] accession number
Antrodia xantha	–	AJ006681
Antrodiella onychoides	–	AJ006674
A. semisupina	–	AJ006675
Bjerkandera adusta	–	AJ006672
B. furnosa	–	AJ006673
Ceriporiopsis aneirina	FP-104462-sp	AY219362
C. gilvescens	–	AJ006684
C. pannocincta	FP-100624-sp	AY219361
C. rivulosa	JLL-10602-sp	AY219363
Diplomitoporus lindbladii	–	AJ006682
Irpex lacteus	–	AB079266
Leptoporus mollis	–	AJ006669
Phanerochaete allantospora	KKN-111–sp	AY219357
P. arizonica	RLG-10816-sp	AY219350
P. avellanea	FP-104126-sp	AY219355
P. australis (1)	FP-102818-sp	AY219373
P. australis (2)	FP-102907-sp	AY219374
P. australis (5)	HHB-7105-sp	AY219371
P. australis (7)	HHB-7083-sp	AY219372
P. burtii	FP-104384-sp	AY219352
P. carnosa	HHB-10118-sp	AY219354
P. chrysorhiza	FP-102002-sp	AY219359
P. chrysosporium	BKM-F-1767	AY219344
P. crassa	FP-102496-sp	AY219341
P. ericina	FP-101978-sp	AY219345
P. filamentosa	FP-105240-sp	AY219340
P. flava	PR-3147	AY219358
P. gigantea [c]	–	AF087488
P. hiulca	FP-100589-sp	AY219342
P. laevis (1)	FP-101481-sp	AY219347
P. laevis (2)	FP-101018-sp	AY219348
P. magnoliae	HHB-9829-sp	AY219343
P. 'pseudomagnoliae'	PP25	AY219370
P. omnivora	HHB-5969-sp	AY219360
P. radicata	HHB-1909-sp	AY219339
P. rimosa	FP-102099-sp	AY219349
P. sanguinea	FP-100391-sp	AY219353
P. sordida (1)	HHB-7423-sp	AY219375
P. sordida (2)	FP-133262-sp	AY219376
P. sordida (3)	HHB-7827-sp	AY219377
P. sordida (4)	HHB-11458-sp	AY219378
P. sordida (6)	HHB-7201-sp	AY219379
P. sordida (8)	HHB-9702-sp	AY219380
P. sordida (14)	HHB-9650-sp	AY219381
P. sordida (15)	HHB-9899-sp	AY219382
P. sordida (16)	HHB-8122-sp	AY219383
P. sordida (17)	HHB-10007-sp	AY219384
P. sordida (20)	HHB-9871-sp	AY219385
P. subceracea	FP-105974-sp	AY219346
P. tuberculata	FP-102168-sp	AY219356
P. velutina	FP-102157-sp	AY219351
Phlebia albida	GB-1833	AY219368
P. albomellea	FP-101843-sp	AY219369
P. chrysocreas	FP102161-sp	AY219367
P. concentrica	OSC-41587-sp	AY219364
P. radiata	JLL-15608-sp	AY219366
P. subserialis	GB-240	AY219365
Spongipellis delectans	–	A5006670
S. spumeus	–	A5006671
Tyromyces alborubescens [d]	–	AJ006683

[a] Strain numbers of Center for Forest Mycology Research, USDA Forest Products Laboratory, Madison, WI.

[b] National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/, last accessed on 9 Oct. 2002).

[c] Originally submitted to GenBank as *Phlebiopsis*.

[d] Orignially submitted to GenBank as *Aurantiporus*.

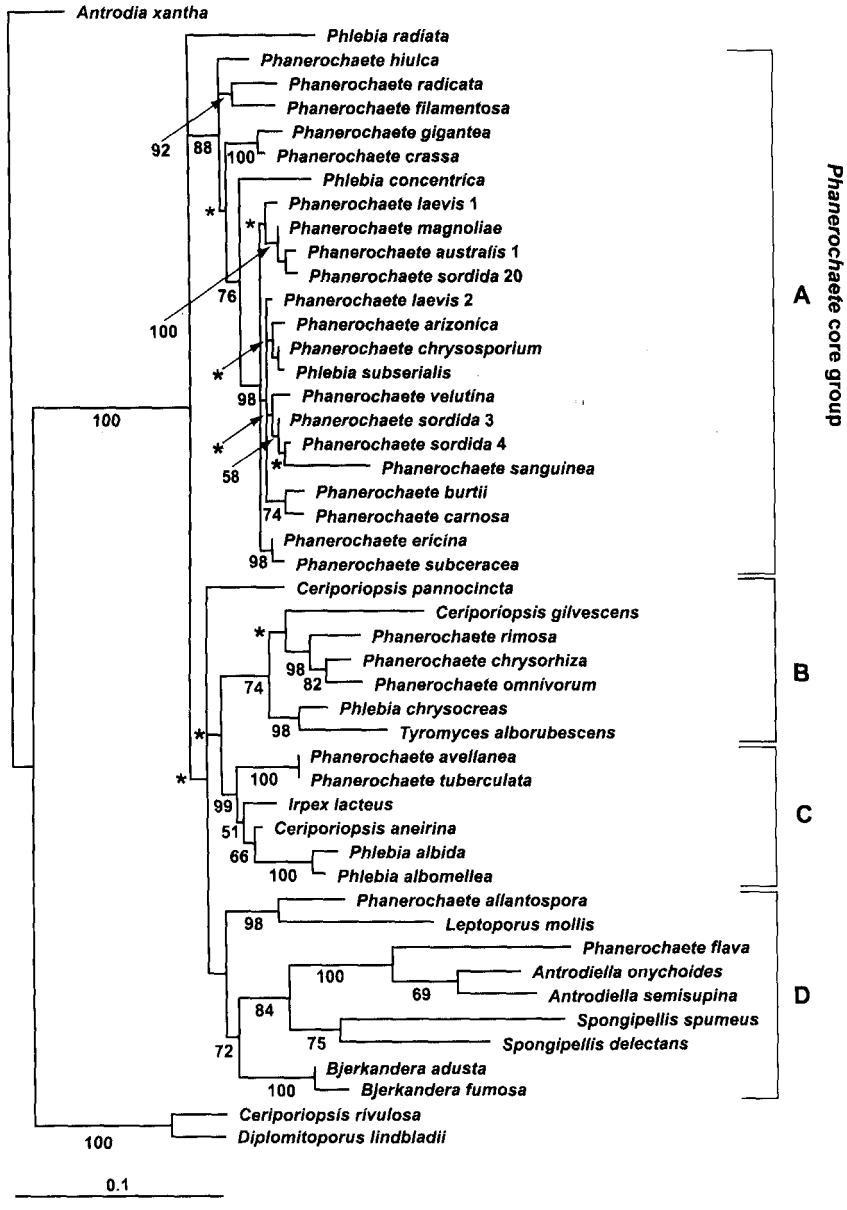

Fig. 1. Maximum likelihood tree based on ITS sequences of *Phanerochaete*, allied and outgroup taxa. *, branches that collapsed in the Bayesian analysis, numbers are averaged values from three Bayesian analyses. Clade A, core *Phanerochaete* group; clades B, C and D, see text for discussion.

Mountain View, CA). Sequences generated from this study were submitted to GenBank (accession nos. AY219339–AY219385) and are listed in Table 1.

Phylogenetic analysis

Sequences of the ITS region, obtained from this study and GenBank (Table 1), were aligned manually, in McClade version 3 (Maddison & Maddison 1992) and PAUP* 4.0b10 (Swofford 2002). Gaps were introduced into the sequences to increase their aligned similarity. Two datasets were created and analysed. The first dataset of 48 taxa includes representatives of each *Phanerochaete* species (24 taxa), allied (17), and outgroup taxa (7). The second dataset includes the *Phanerochaete* taxa in the monophyletic clade A (Fig. 1) as well as additional strains of *P. sordida* and *P. pseudomagnoliae.* Sequences in the second dataset were realigned, and ambiguous sites were excluded before analyses. Phylogenetic analyses of the sequence data were performed with maximum parsimony (MP) and maximum likelihood (ML) methods as implemented in PAUP* 4.0b10 and with Bayesian inference using MrBayes version 2.01 (Huelsenbeck & Ronquist 2000). Aligned sequence data matrices and trees are deposited at TreeBASE (PIN 11824, http://www.treebase.org/).

For MP analyses, an initial heuristic search of 100 random taxon addition replicate searches was conducted with TBR branch-swapping, MAXTREES set to autoincrease (exceptions as discussed in Results), without constraints, all characters unordered and equally weighted, gaps treated as missing data, and with two trees held during each stepwise addition cycle. The shortest trees from this analysis were used as

starting trees in a second heuristic search, with the same parameters described above, to find the most parsimonious trees.

The best model of sequence evolution was determined using nested likelihood ratio tests calculated with Modeltest 3.06 (Posada & Crandall 1998). The values obtained from Modeltest were then used in ML and Bayesian analyses. Maximum likelihood (ML) heuristic searches were performed in PAUP* 4.0b10 with TBR branch swapping. Bayesian analysis was implemented using MrBayes starting with a random tree, running four simultaneous Monte Carlo chains, with no molecular clock enforced. One million generations were performed, with every 100 trees sampled. The first 10% of the trees were excluded from construction of the consensus tree. Bayesian analyses were performed three times to confirm the consistency of the consensus tree and to calculate the posterior clade probability averages.

To test for the monophyly of the genus *Phanerochaete,* we conducted ML searches in which all the *Phanerochaete* species were Constrained in a clade. In addition, since *P. rimosa* is sometimes placed in a separate genus, *Scopuloides* (Jülich 1982), a separate ML search was done with *P. rimosa* removed from the constrained group. The constrained trees were compared with the unconstrained tree using the Shimodaira–Hasegawa (SH) test as implemented in PAUP* 4.0b10 (Goldman, Anderson & Rodrigo 2000, Shimodaira & Hasegawa 1999). In the analysis, 1000 bootstrap replicates were performed with the re-estimated log likelihood (RELL) option.

RESULTS

Sequence data and alignment

The size of the ITS region in all the taxa was about 600 base pairs (bp). The 5.8 S rDNA sequences were 133–135 bp long and nearly identical for all the taxa. The size of ITS1 spacer region for the *Phanerochaete* taxa ranged from 201 to 246 bp and those of ITS2 spacer region from 178 to 218 bp. Variability consisted of single base changes or single base insertions as well as areas of larger insertions and/or deletions. Areas with larger insertions or deletions were difficult to align and were excluded from phylogenetic analysis.

Analysis of Dataset 1

Alignment of ITS dataset 1 included 48 sequences representing 24 *Phanerochaete* taxa and 24 allied and outgroup taxa. The alignment totalled 905 nucleotide positions after the addition of gaps to improve the alignment. Areas of ambiguous alignment were excluded leaving 370 characters of which 222 were constant, 37 were parsimony uninformative, and 111 were parsimony informative. Modeltest selected the transitional model with equal base frequencies and gamma distributed site-to-site rate variation (TIM + I + G) as the best-fit model for explaining evolutionary change within the selected taxa. Fig. 1 shows the unconstrained ML tree rooted with *Antrodia xantha.* Four distinct clades (labelled A–D) are present in this tree with species of *Phanerochaete* distributed in all four clades. Clade A is composed of the core *Phanerochaete* group including two *Phlebia* species. This clade was selected for further analysis (Dataset 2). *Phanerochaete rimosa, P. chrysorhiza, P. omnivora, P. avellanea, P. tuberculata, P. Java,* and *P. allantospora* did not cluster with the core *Phanerochaete* species in clade A. Within clade B, *P. rimosa, P. chrysorhiza* and *P. omnivorum* clustered together. Other taxa in clade B included *Phlebia chrysocreas, T. alborubescens,* and *Ceriporiopsis gilvescens. Phanerochaete avellanea* and *P. tuberculata* formed a strongly supported subclade at the base of clade C that also included *Irpex lacteus, Phlebia albida, Phlebia albomellea,* and *C. aneirina.* In clade D, *P. allantospora* clusters with *Leptoporus mollis* whereas *P. flava* is in a group with *Antrodiella semisupinus* and *A. onychoides.* The Bayesian trees (not shown) are similar to the ML tree but less resolved; branches that collapse in the Bayesian analyses are indicated by asterisks in Fig. 1.

Maximum parsimony (MP) analysis, with Maxtrees limited to 2000, yielded 1200 most parsimonious trees. The MP trees were 479 steps long, and excluding uninformative characters, the following values were obtained: C.I.=0.395, R.I.=0.616, and R.C.=0.275. The 50% majority rule consensus MP tree (data not shown) is basically congruent with the ML tree except for *Phlebia radiata,* which clusters with taxa in clade B, and *L. mollis* and *P. allantospora* that drop out of clade D.

The monophyly of *Phanerochaete* was not supported (Table 2). The Shimodaira–Hasegawa test showed that the best tree was the unconstrained ML tree. The ML constrained trees, including and excluding *P. rimosa,* were significantly more unlikely than the unconstrained tree (Table 2).

Table 2. Results of Shimodaira–Hasegawa lest (Shimodaira & Hasegawa 1999).

Tree	–ln L	Diff. – ln L
Unconstrained tree	2863.1	–
Constrained tree with *P. rimosa*[a]	2970.8	107.7[b]
Constrained tree without *P. rimosa*	2976.8	113.7[b]

[a] This tree is forces all *Phanerochaete* species into a monophyletic clade.

[b] Significant at the 0.05 level.

Analysis of Dataset 2 (core Phanerochaete *group)*

In dataset 2, the core *Phanerochaete* group (clade A, Fig. 1) was analysed further. *Phanerochaete pseudomagnoliae* as well as additional representatives of *P. sordida* were included in the analysis. To simplify the analysis, *P. sordida* and *P. australis* isolates were placed in groups based on overall sequence similarity.

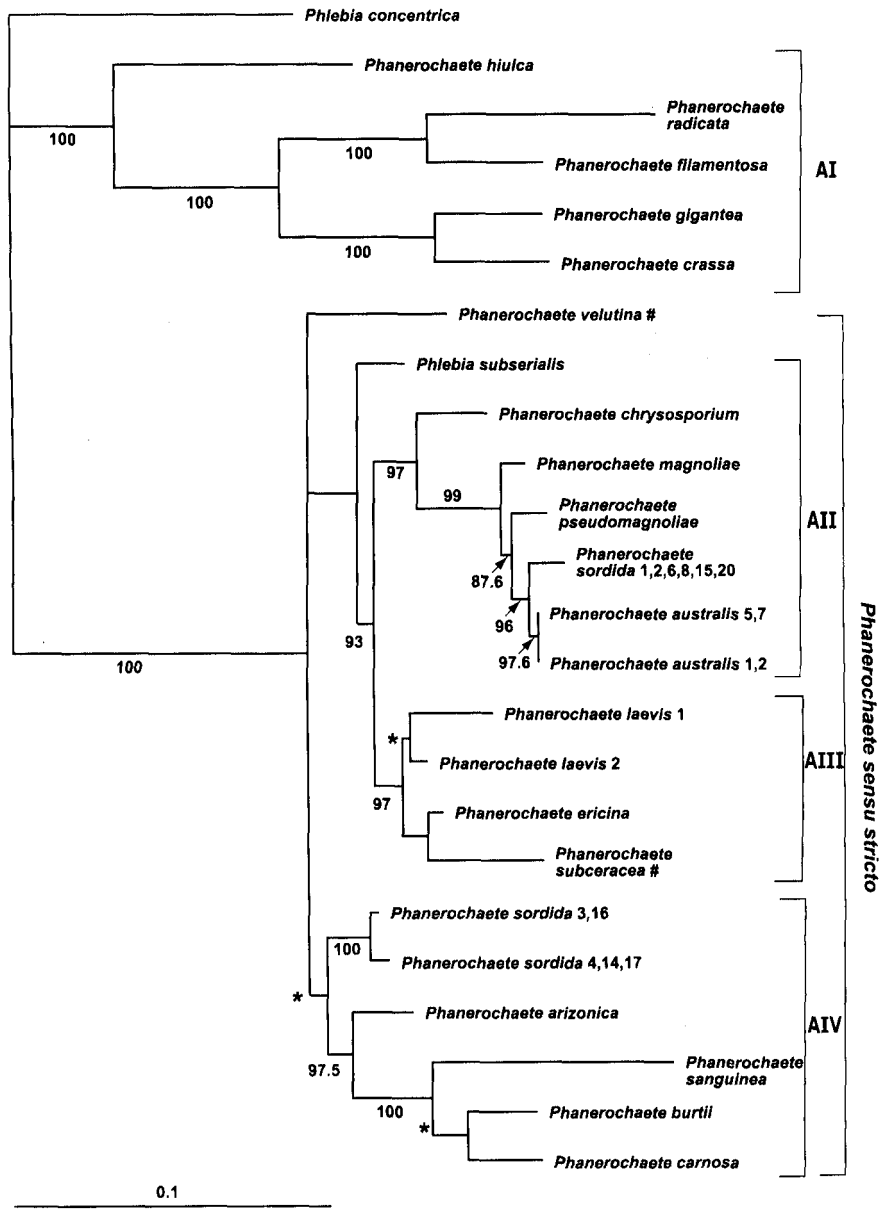

Fig. 2. Maximum likelihood tree based on ITS sequences of clade A, *Phanerochaete* core group (see Fig. 1). *, branches that collapsed in the Bayesian analysis, numbers are averaged values from three Bayesian analyses; #, taxa that form a clade in Bayesian analysis.

The difference in sequence within each group was less than 1%; thus, only one isolate was included in the analysis to represent the group. Two species of *Phlebia, viz. Phlebia subserialis* and *Phlebia concentrica,* were included in this dataset because they were embedded within clade A (Fig. 1). Dataset 2 included ITS sequences of 24 isolates representing 19 *Phanerochaete* species rooted with *Phlebia concentrica.* There were 777 nucleotide positions, after the addition of gaps to improve the alignment. After areas of ambiguous alignment were excluded, 500 characters remained of which 328 were constant, 60 were parsimony uninformative, and 112 were parsimony informative. Modeltest selected the transitional model with equal base frequencies and gamma distributed site-to-site rate variation (TrN + G) as the best-fit model for this dataset.

Fig. 2 shows the ML tree of the core *Phanerochaete* species of clade A, including all duplicate isolates. Four distinct clades (labelled AI–AIV), separated into two major groups, are present in this tree. The smaller group, clade AI, contains *P. hiulca, P. radicata, P. filamentosa, P. gigantea* and *P. crassa. Phanerochaete sordida* isolates are divided between clades AII and AIV. Within clade AII, *P. sordida* (isolates 1–2, 6, 8, 15, and 20) and *P. australis* form a strongly supported clade. The other isolates of *P. sordida* (isolates 3–4, 14, 16, and 19) are weakly aligned with *P. arizonica, P. sanguinea, P. burtii,* and *P. carnosa* in clade AIV. Bayesian trees (not shown) are similar to the ML tree

Table 3. Distribution of species within phylogenetic clades based on ITS sequences and morphologically-based subgenera according to Burdsall (1985) or other genera.

Clade[a]	Subgenera			Other genera microscopic characters variable
	Phanerochaete cystidia present	*Phanericium* cystidia & pseudocystidia absent	*Scopuloides* cystidia absent; pseudocystidia present	
AI	*Phanerochaete filamentosa* *P. radicata*		*P. hiulca* *P. gigantea* *P. crassa*	
AII	*P. magnoliae* *P. australis* (1, 2, 5, 7) *P. sordida* (1, 2, 6, 8, 15, 20) *P. chrysosporium* *P. 'pseudomagnoliae'*			*Phlebia concentrica* *P. subserialis*
AIII	*P. laevis* (1, 2) *P. ericina* *P. subceracea* *P. velutina*[b]			
AIV	*P. sordida* (3, 4, 14, 16, 17) *P. arizonica* *P. sanguinea* *P. burtii* *P. carnosa*			
B	*P. chrysorhiza* *P. omnivora*		*P. rimosa*	*Ceriporiopsis pannocincta* *C. gilvescens* *P. chrysocrea* *Tyromyces alborubescens*
C		*P. avellanea* *P. tuberculata*		*Irpex lacteus* *C. aneirina* *P. albida* *P. albomellea*
D	*P. allantospora* *P. flava*			*Leptoporus mollis* *Antrodiella onychoides* *A. semisupinia* *Spongipellis spumeus* *S. delectans* *Bjerkandera adusta* *B. fumosa*

[a] Clade designations from Figs 1–2.
[b] The position of *P. velutina* is unstable; it is in clade AIII or AIV.

but slightly less resolved. In Fig. 2 branches that collapse in the Bayesian analyses are indicated by asterisks, and *P. velutina* and *P. subceracea* (indicated by hash marks) formed a separate clade.

Maximum parsimony analysis yielded 8 most parsimonious trees. The MP trees were 355 steps long, and excluding uninformative characters, with C.I. = 0.567, R.I. = 0.677, and R.C. = 0.444. The 50% majority rule consensus tree (not shown) is basically congruent with the ML tree, except that the *P. sordida* isolates in clade AIV formed a separate clade with *P. velutina.*

DISCUSSION

The ITS region is useful for inferring phylogenetic relationships among closely related *Phanerochaete* species. Although the 5.8 S ribosomal RNA region was highly conserved among all the taxa studied, there were significant differences in the length and sequence of the flanking ITS regions. Maximum likelihood and maximum parsimony analyses produced largely congruent trees and indicated that *Phanerochaete*, as presently defined, is a polyphyletic genus. In addition, ITS data support the observation that *P. sordida* represents a species complex of two morphologically similar but genetically different taxa (Eriksson *et al.* 1978, Burdsall 1985).

Although the difficulty of reconciling phylogenetic clades with classification ranks is well recognized (Hibbett & Donoghue 1998), clade A, the *Phanerochaete* core group of Fig. 1, agreed reasonably well with Burdsall's (1985) *Phanerochaete* subgen. *Phanerochaete* except for the inclusion of *Phlebia subserialis* and *P. concentrica* (Table 3). Previous phylogenetic studies using the ITS region also show *P. subserialis* clustered with *Phanerochaete* species instead of *Phlebia* species (Boidin *et al.* 1998, Lim 2001). However, analyses of small and large subunit ribosomal RNA gene sequences placed *P. subserialis* in a clade with other *Phlebia* species (Parmasto & Hallenberg 2000, Moncalvo *et al.* 2002, Suhara *et al.* 2002). Similarly, basidiome and cultural morphologies do not support

a close relationship of *Phlebia subserialis* to *Phanerochaete*. Thus, the placement of *Phlebia subserialis,* and by extension *P. concentrica,* in the core *Phanerochaete* group by ITS data may be a result of the peculiarities of the region that reflect the phylogenetic relationships of that region but not of the species as a whole.

Further analysis of clade A, the core *Phanerochaete* group, produced four distinct subclades, AI to AIV (Fig. 2, Table 3). Clade AI is sister to the *Phanerochaete s. str.* group. In clade AI, *P. gigantea, P. hiulca,* and *P. crassa* are classified in subgenus *Scopuloides* whereas *P. flamentosa* and *P. radicata* are in subgenus *Phanerochaete*. Some of these taxa have been placed in other genera. For example, *P. gigantea* was placed in *Phlebia* (Donk 1957) and *Phlebiopsis* (Jülich 1978) based on morphological features. Recent molecular phylogenetic studies using various regions of the nuclear ribosomal DNA region have been unable to resolve the placement of *P. gigantea.* One study supports its placement in *Phlebia* (Parmasto & Hallenberg 2000), whereas our results and other studies support a closer relationship to *Phanerochaete* (Kim & Jung 2000, Lim 2001, Hibbett & Binder 2002, Moncalvo *et al.* 2002). Similarly, *P. crassa* was transferred to the genus *Porostereum* because of its pigmented cystidia and dimitic hyphal system (Hjortstam & Ryvarden 1990). *Phanerochaete radicata* and *P. flamentosa* of subgen. *Phanerochaete* are closely related taxa (Nakasone, Bergman & Burdsall 1994) that are morphologically distinct from other species of *Phanerochaete.* They are characterized by pellicular fruit bodies that stain red in 1% potassium hydroxide, hyphal strands, and small ellipsoid basidiospores. Lim (2001) reported similar results in that *P. gigantea* and *P. crassa,* as well as *P. xerophila, Cystidiophorus castaneus, Australohydnum dregeanum,* and other taxa, belonged in a clade that was sister to a group of primarily *Phanerochaete* species.

Phanerochaete s. str. *group (clades AII, AIII, AIV)*

Phanerochaete velutina, clades AII (excluding *Phlebia subserialis),* AIII, and AIV, constitute the *Phanerochaete s. str.* group (Fig. 2). All the species in this group are classified in subgenus *Phanerochaete,* and they all develop hymenial cystidia and small, ellipsoid basidiospores. The type species of *Phanerochaete* is *P. velutina* but its placement could not be firmly established by ITS data. In ML trees, *P. velutina* is not associated with any of the three subclades of the *Phanerochaete s. str.* group, but in Bayesian analyses, it forms a clade with *P. subceracea.*

Clade AII is a strongly supported clade composed of five *Phanerochaete* species. *Phanerochaete chrysosporium, P. magnoliae* and *P. pseudomagnoliae* develop smooth, cylindrical to slightly clavate cystidia with obtuse apices in contrast to the often encrusted, pyriform to cylindrical cystidia with tapering apices of *P. sordida* and *P. australis.*

The species in clade AIII are characterized by light brown, brownish orange, or greyish yellow to greyish orange fruitbodies and slender, encrusted cystidia that taper toward the apex. in addition, *P. ericina* and *P. subceracea* have a dimitic hyphal system in which generative and narrow, much branched binding-type hyphae are produced.

Two weakly supported subclades comprise clade AIV. Five of the *P. sordida* isolates make up one of the subclades. This group is genetically distinct from the *P. sordida* isolates in clade AII. Preliminary microscopic observations also indicate morphological differences between these two *P. sordida* groups, e.g. cystidia are embedded in clade AII but not in clade AIV. A thorough morphological study of the type specimen of *P. sordida* and all synonyms is required to determine the correct names for these two groups. The other subclade contains *P. arizonica, P. burtii, P. carnosa* and *P. sanguinea.* These species are characterized by yellowish or reddish fruit bodies with fibrillose margins, with or without hyphal strands, and slender, smooth or encrusted, cylindrical cystidia tapering toward the apex.

Phanerochaete *species in clades B, C, and D*

Seven *Phanerochaete* species did not cluster with the *Phanerochaete* core group based on phylogenetic analyses of the ITS region. These taxa are discussed below. Our results suggest that these taxa are only distantly related to *Phanerochaete* adn should be transferred to other genera. However, we believe that not enough data are available at this time to warrant any nomenclatural changes. Additional molecular data from other gene regions are necessary to confirm the results from the ITS region presented here. Furthermore. a thorough morphological study of the taxa is necessary, for many of these non-core *Phanerochaete* species do not have any readily apparent phenotypic features that would justify their removal from *Phanerochaete.*

In clade B (Fig. 1, Table 3) *P. rimosa, P. chrysorhiza,* and *P. omnivora* form a strongly supported subclade. *Phanerochaete rimosa,* of subgen. *Scopuloides,* is generally accepted in *Scopuloides* (Jülich 1982), a genus characterized by ceraceous to crustaceous, spine-bearing fruit bodies, conical, encrusted cystidia, and small allantoid basidiospores. Our results indicate that Scopuloides is the correct generic placement of P. rimosa. Although *P. chrysorhiza* and *P. omnivora* (subgen. *Phanerochaete*) also develop spines, they otherwise bear no resemblance to *P. rimosa.* Their brightly pigmented, yellow or orange, membranous spinose fruit bodies, fibrillose margins with hyphal strands. and small, hyphoid to ventricose, smooth cystidia assist in their identification. *Phanerochaete chrysorhiza* and *P. omnivora* also clustered with *Phlebia chrysocreas,* T. alborubescens, and C. gilvescens as first demonstrated by Lim (2001). Other molecular phylogenetic

studies have shown also that *P. chrysorhiza* is distantly related to the core *Phanerochaete* species of clade A (Hibbett & Binder 2002, Moncalvo *et al.* 2002).

P. avellanea and *P. tuberculata,* members of clade C, form a strongly supported subclade. Burdsall (1985) placed them in subgen. *Phanericium,* taxa that lack cystidia and pseudocystidia (Table 3). They are sister to a clade that contains. *Irpex lacteus, C. aneirina, Phlebia albida* and *Phlebia albomellea.* Lim (2001), using the same ITS region, also showed *P. tuberculata* in a clade with *I. lacteus.* Because only two authenticated species of subgen. *Phanericium* were available for this study, no conclusions can be made about the taxonomic validity of the subgenus. Future phylogenetic studies of *Phanerochaete* should include more authenticated species of subgen. *Phanericium.*

In clade D, two species of *Phanerochaete* subgen. *Phanerochaete* are clustered with species of *Bjerkandera, Spongipellis, Antrodiella,* and *Leptoporus. P. flava* is joined in a strongly supported clade with *A. onychoides* and *A. semisupina.* However, there are no morphological traits that would justify the removal of *P. flava* from *Phanerochaete* at this time. In another strongly supported clade, *P. allantospora* pairs with *L. mollis. P. allantospora* produces large, allantoid basidiospores that are unique in the genus, and this may be sufficient to remove the taxon from *Phanerochaete.*

Our ITS data also suggest that *Ceriporiopsis* and *Phlebia* are not monophyletic genera. Four taxa of *Ceriporiopsis* and six taxa of *Phlebia* are placed in four clades each (Fig. 1). *Ceriporiopsis* is recognized as a heterogeneous assemblage of taxa of unrelated phylogenetic origins (Ryvarden & Gilbertson 1993). Similarly, *Phlebia* is a collection of unrelated taxa that have some common morphological traits (Eriksson, Hjortstam & Ryvarden 1981). Several molecular phylogenetic studies have demonstrated that *Phlebia* is not monophyletic (Parmasto & Hallenberg 2000, Moncalvo *et al.* 2002).

In this preliminary molecular phylogenetic study, we demonstrate that *Phanerochaete* as presently defined is not a monophyletic genus. A core group of *Phanerochaete* species emerged that more or less corresponds to subgen. *Phanerochaete* as defined by Burdsall (1985). These taxa develop true hymenial cystidia, and *P. velutina,* the type of the genus, is included in this core group. Seven *Phanerochaete* species, however, were clearly shown to have closer affinities with other polyporoid lineages; this indicates that convergent morphology to a phanerochaetoid type has occurred multiple times.

A revised definition of the genus *Phanerochaete* will require a multi-faceted approach that encompasses morphological, cultural, physiological, chemical, ultrastructural as well as molecular data. Chemical properties such as laccase and tyrosinase spots tests have been suggested to be of taxonomic value (Marr, Grund & Harrison 1986). These and other tests could be used to enhance the classification of *Phanerochaete* species that are currently based on morphological properties. Additional taxa from subgens *Phanericium* and *Scopuloides* should be included in future analyses to remove bias toward subgenus *Phanerochaete.* Although ITS sequences are a valuable tool to infer the phylogenetic relationships among species of *Phanerochaete s. str.* and to distinguish new species, other non-protein and protein coding gene regions should be employed to fully resolve ambiguities, notably the placement of *P. velutina, P. hiulca, Phlebia subserialis* and *Phlebia concentrica,* and the phylogenetic position of *Phlebiopsis gigantea.*

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