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Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus†

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Stachybotrys chartarum is an asexually reproducing fungus commonly isolated from soil and litter that is also known to occur in indoor environments and is implicated as the cause of serious illness and even death in humans. Despite its economic importance, higher level phylogenetic relationships of *Stachybotrys* have not been determined nor has a sexual state for *S. chartarum* been reported. DNA sequences from four nuclear and one mitochondrial gene were analyzed to determine the ordinal and familial placement of *Stachybotrys* within the *Euascomycota*. These data reveal that species of *Stachybotrys* including *S. chartarum*, *S. albipes*, for which the sexual state *Melanopsamma pomiformis* is reported, species of *Myrothecium*, and two other tropical hypocrealean species form a previously unknown monophyletic lineage within the *Hypocreales*. These results suggest that *Stachybotrys* and *Myrothecium* are closely related and share characteristics with other hypocrealean fungi. In addition, *S. chartarum* may have a sexual state in nature that consists of small, black, fleshy perithecia similar to *Melanopsamma*.

INTRODUCTION

Stachybotrys chartarum is an asexually reproducing fungus that is commonly isolated from soil, leaf litter, and dung from throughout the world (Domsch, Gams & Anderson 1980, Koster *et al.* 2003). In recent years this species has achieved notoriety as an airborne house-inhabiting fungus that produces mycotoxins and can cause serious illness and even death in humans (Vesper *et al.* 2000). Considerable effort is given to monitoring the presence of this fungus particularly in situations where building interiors, especially substrates rich in cellulose, have become wet, thus supporting the growth of this and other mycotoxin-producing molds (Andersen, Nielsen & Jarvis 2002, Shelton *et al.* 2003, Spurgeon 2003).

A sexual state for *S. chartarum* has never been reported. As an asexually reproducing fungus, *S. chartarum* is, at least theoretically, a clonal organism. An increasing body of evidence suggests that, despite the lack of a sexual state, a number of fungi known to reproduce only asexually appear to be undergoing the equivalent of sexual reproduction resulting in genetically diverse populations (Taylor, Jacobson & Fisher 1999, Taylor *et al.* 2000, Bidochka & Koning 2001). In addition, many asexually reproducing fungi are derived from within groups that include sexually reproducing species (O'Donnell, Cigelnik & Nirenberg 1998, Chaverri *et al.* 2003). Only one species of *Stachybotrys*, *S. albipes*, has been linked to a sexual state reported as *Melanopsamma pomiformis* (Booth 1957), although this research has not been repeated. *M. pomiformis* is a small black perithecial ascomycete less than 300 μm diam that occurs on decorticated rotten wood, especially hardwoods (Booth 1957, Samuels & Barr 1997). This species is widely distributed throughout the north temperate regions of the world (Samuels & Barr 1997, Farr *et al.* 2004) including a report of the asexual state from black rush, *Juncus roermerius*, in salt marshes (Fell & Hunter 1979). The genus *Melanopsamma* has been placed in an obscure family, *Niessliaceae*, in the *Hypocreales* (Samuels & Barr 1997, Rossman *et al.* 1999).

Intrageneric relationships within *Stachybotrys* and the synonymous genus *Memnoniella* have been investigated (Haugland, Vesper & Harmon 2001) and species level studies of *S. chartarum* have resulted in the description of a new species and the presence of

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two chemotypes in *S. chartarum* (Andersen *et al*. 2002, 2003, Cruse *et al*. 2002). Despite its economic importance, higher level phylogenetic relationships of the genus *Stachybotrys* have not been investigated. To determine if *S. chartarum* is derived from within the *Hypocreales*, we sequenced both the nuclear encoded small subunit ribosomal DNA (nrSSU) and the nuclear encoded large subunit ribosomal DNA (nrLSU) for isolates of *Stachybotrys*, including *S. chartarum*, and analyzed them with major orders of the *Sordariomycetes*. To determine family placement within the *Hypocreales*, we sequenced regions of RNA polymerase II largest subunit (RPB1), translation elongation factor 1-α (EF1-α) and mitochondrial ATP synthase 6 (ATP6) and analyzed them in combination with the ribosomal RNA genes for representatives of the known families of the *Hypocreales*.

MATERIAL AND METHODS

Nucleic acid extraction and PCR amplification

Culture or specimen numbers for species used in this study are listed in Table 1. Mycelia for DNA extractions were grown on PDA plates, scraped from the plates with a sterile scalpel, placed in 1.7 ml tubes and extracted using the PureGene DNA Extraction Kit (Gentra Systems, Minneapolis, MN) according to the manufacturer's instructions.

Individual genes were amplified in 50 μl reactions on a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA) and or I-Cycler (Bio-Rad, Hercules, CA) using the following previously published primers: nrLSU, LR0R and L7 (Vilgalys & Hester 1990, Rehner & Samuels 1994); nrSSU, NS1 and NS8 (White *et al*. 1990); EF1-α, EF1-983f and EF1-2218r (Rehner 2001, Currie *et al*. 2003); and RPB1Cr (Hall 2003). The following primers were designed by G.-H.S. for use with hypocrealean taxa: RPB1 forward primers, CRPB1 (CCWGGYTTYATCAAGAARGT) and CRPB1A (CAYCCWGGYTTYATCAAGAA) and ATP6 forward and reverse primers, ATP6-C1A (AGAWCAATTYGAARTRAGAG) and ATP6-C2A (ACAAAYACTTGWGCTTGKATWAAIGC). Failsafe PCR master mix B (Epicentre, Madison, WI) was used according to the manufacturer's instructions for amplifications that were problematic.

Standard cycling parameters with a 55 °C annealing temperature were used for nr-SSU, nr-LSU, and EF1-α. For the RPB1 and ATP6 genes the thermal cycler program was as follows: (1) 1 min at 94 °, 35 s at 37 °, 1 ° every 4 s to 72 °, 1 min at 72 ° run for four cycles; (2) 35 s at 94 °, 55 s at 45 °, 1 ° every 4 s to 72 °, 1 min at 72 °, 29 cycles; and (3) final extension 10 min at 72 °. The PCR products were purified using ExoSAP-IT (USB, Cleveland, OH) according to the manufacturer's instructions. Amplified products were sequenced with the BigDye version 3.1 dye terminator kit (Applied Biosystems) on an ABI 3100 automated DNA sequencer. The PCR primers were used as sequencing primers for all genes. The following internal primers were used for sequencing nrSSU, in addition to the PCR primers listed previously: NS2, NS3, NS4, NS5, NS6, NS7 (White *et al*. 1990). The following internal primers were used for sequencing nrLSU in addition to the previously mentioned PCR primers: LR3R and LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1995).

A few genes were unable to be sequenced for certain isolates (Table 1) and were treated as missing data in the analyses. For RPB1, the primer CRPB1A rather than CRPB1 was used for the following four isolates: *Myrothecium leucotrichum* CBS 114052, *Ochronectria calami* CBS 125.87, *Peethambara spirostriata* CBS 110115, and *Stilbocrea macrostoma* GJS 73-26. Sequences were deposited in GenBank and listed in Table 1.

Phylogenetic analyses

Maximum parsimony (MP) analyses were conducted using PAUP 4.0b10 (Swofford 2002) and Bayesian analyses were conducted with MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001) for each alignment. MP analyses were conducted heuristically with 1000 random taxon addition replicates with TBR-branch swapping and MULTREES on. 1000 bootstrapping pseudoreplicates with ten random taxon addition replicates per pseudoreplicate were performed. The Bayesian analyses employed the GTR + Γ + I model and included three separate runs each consisting of 500 000 Markov Chain Monte Carlo (MCMC) generations, each with a burn-in of 100 000 generations. Posterior probabilities from the three runs were pooled and indicated on Figs 1–2. Alignment 1 was derived from Kohlmeyer, Spatafora & Volkmann-Kohlmeyer (2000), with the addition of sequences from *Melanospora* and *Sphaerodes* (Zhang & Blackwell 2002) and the *Diaporthales* (Castlebury *et al*. 2001) as well as newly sequenced species from this study. The alignments have been deposited in TreeBASE.

Two datasets were analyzed as follows: (1) an alignment of nrSSU and nrLSU sequences for the major orders of the *Sordariomycetes*, including representative taxa within *Stachybotrys*, *Myrothecium* and known hypocrealean families, with the *Pezizomycetes* as outgroup taxa to determine if *S. chartarum* belongs within the *Hypocreales*; and (2) an alignment of the nrSSU, nrLSU, EF1-α, ATP6, and RPB1 for an expanded set of taxa from *Stachybotrys*, *Myrothecium* and families within the *Hypocreales*, using *Glomerella cingulata* and *Verticillium dahliae* (*Phyllachorales*) as outgroup taxa, in order to determine the closest relatives of *S. chartarum* and related species.

RESULTS

Alignment 1 consisted of nrSSU (1024 bp) and nrLSU (596 bp) sequences for 67 taxa. Of the total of 1620

Table 1. List of taxa sequenced in this study.

Taxon	Source[a]	Habitat or host	LSU	SSU	EF1	ATP6	RPB1
Aphysiostroma stercorarium	ATCC 62321 ex-type	Spain, on cow dung	AF543792	AF543769	AF543782	AY489566	AY489633
Balansia henningsiana	GAM 16112	Georgia, on *Panicum* sp.	AY489715	AY489683	AY489610	AY489576	AY489643
Bionectria ochroleuca	CBS 114056	Venezuela, on bark	AY489176	AY489684	AY489611	–	–
B. pityrodes	ATCC 208842	Mauritius, on bark	AY489728	AY489696	AY489623	AY489589	AY489658
Claviceps purpurea	GAM 12885	Georgia, on *Dactylis glomerata*	AF543789	AF543765	AF543778	–	AY489648
Cordyceps capitata	OSC 71233	France, on *Elaphomyces* sp.	AY489721	AY489689	AY489615	AY489581	AY489649
C. gunnii	OSC 76404	Australia, on Lepidoptera	AF339522	AF339572	AY489616	AY489582	AY489650
C. heteropoda	OSC 106404	Australia, on Hemiptera	AY489722	AY489690	AY489617	–	AY489651
C. ophioglossoides	OSC 106405	North Carolina, on *Elaphomyces* sp.	AY489723	AY489691	AY489618	AY489583	AY489652
Cosmospora coccinea	CBS 114050	Germany, on *Inonotus nodulosus*	AY489734	AY489702	AY489629	AY489596	AY489667
Didymostilbe echinofibrosa	AR 2824	Mexico, isol. from litter	AY489706	AY489674	AY489601	AY489567	AY489634
Epichloe typhina	ATCC 56429	New Zealand, on *Festuca rubra*	U17396	U32405	AF543777	AY489584	AY489653
Glomerella cingulata	CBS 114054	Florida, isol. from *Fragaria*	AF543786	AF543762	AF543773	AY489590	AY489659
Haematonectria haematococca	CBS 114067	Guyana, on bark	AY489729	AY489697	AY489624	–	AY489660
Hydropisphaera peziza	CBS 102038	Alabama, on bark	AY489730	AY489698	AY489625	AY489591	AY489661
Hypocrea rufa	CBS 114374	North Carolina, on bark	AY489726	AY489694	AY489621	AY489587	AY489656
H. lutea	ATCC 208838	New York, on decorticated conifer wood	AF543791	AF543768	AF543781	AY489592	AY489662
Hypomyces polyporinus	ATCC 76479	North Carolina, on *Trametes versicolor*	AF543793	AF543771	AF543784	AY489593	AY489663
Leuconectria clusiae/ *Gliocephalotrichum bulbilium*	ATCC 22228 ex type	Louisiana, isol. from soil	AY489732	AY489700	AY489627	AY489595	AY489664
Melanopsamma pomiformis/ *Stachybotrys albipes*	ATCC 18873	England, *Ulmus* sp. (single ascospore isolate by C. Booth)	AY489709	AY489677	AY489604	AY489570	AY489637
Myriogenospora atramentosa	AEG 96-32	Georgia, on *Andropogon virginicus*	AY489733	AY489701	AY489628	–	AY489665
Myrothecium cinctum	ATCC 22270	Ukraine, isol. from soil	AY489710	AY489678	AY489605	AY489571	AY489638
M. inundatum	IMI 158855	UK, on *Russula nigricans*	AY489731	AY489699	AY489626	AY489594	–
M. leucotrichum	AR 3506	New Jersey, isol. from decaying grass leaf	AY489707	AY489675	AY489602	AY489568	AY489635
M. roridum	ATCC 16297	Germany, isol. from soil	AY489708	AY489676	AY489603	AY489569	AY489636
M. verrucaria	ATCC 9095	Washington, DC, isol. from baled cotton	AY489713	AY489681	AY489608	AY489574	AY489641
Nectria cinnabarina	CBS 114055	New York, on *Betula*	U00748	U32412	AF543785	–	AY489666
Nectriopsis violacea	CBS 424.64	Germany, on *Fuligo septica*	AY489719	AY489687	–	AY489579	AY489646
Niesslia exilis	CBS 357.70	Germany, on bark of *Picea abies*	AY489718	AY489686	AY489613	AY489578	AY489645
N. exilis	CBS 560.74	UK, on decaying needle of *Pinus sylvestris*	AY489720	AY489688	AY489614	AY489580	AY489647
Ochronectria calami	CBS 125.87	Indonesia, on palm	AY489717	AY489685	AY489612	AY489577	AY489644
Ophionectria trichospora	CBS 109876	Cameroon, on liana	AF543790	AF543766	AF543779	–	AY489669
Peethambara spirostriata	CBS 110115	Ecuador, on brooms of *Crinipellis perniciosa*	AY489724	AY489692	AY489619	AY489585	AY489654
Pseudonectria rousseliana	CBS 114049	Spain, on leaves of *Buxus sempervirens*	U17416	AF543767	AF543780	AY489598	AY489670
Sphaerostilbella berkeleyana	CBS 102308	New Zealand, on polypore	U00756	AF543770	AF543783	–	–

Table 1. (*Cont.*)

Taxon	Source[a]	Habitat or host	LSU	SSU	EF1	ATP6	RPB1
Stachybotrys chartarum	ATCC 9182	Washington DC, isol. from paper	AY489714	AY489682	AY489609	AY489575	AY489642
S. chartarum[b]	ATCC 66238 (=UAMH 6417, =CBS 25089)	Namibia, isol. from desert sand	AY489712	AY489680	AY489607	AY489573	AY489640
S. echinata (=*Memnoniella echinata*)[c]	UAMH 6594	Canada, Alberta, isol. from indoor air	AY489736	AY489704	AY489631	AY489599	AY489672
S. subsimplex (=*M. subsimplex*)	ATCC 32888	Florida, isol. from water hyacinth	AY489711	AY489679	AY489606	AY489572	AY489639
Stephanonectria keithii	CBS 114057	France, on bark of *Eleagnus*	AY489727	AY489695	AY489622	AY489588	AY489657
Stilbocrea macrostoma	CBS 114375	New Zealand, on *Geniostoma ligustifolia*	AY489725	AY489693	AY489620	AY489586	AY489655
Verticillium dahliae	ATCC 16535	Canada, Quebec, isol. from tomato	AY489737	AY489705	AY489632	AY489600	AY489673
Viridispora diparietispora	ATCC MYA 627	New York, on *Crataegus crus-galli*	AY489735	AY489703	AY489630	AY489597	AY489668

[a] ATCC, American Type Culture Collection, Manassas, VA; AR, Amy Y. Rossman personal collection; BPI, US National Fungus Collections, Beltsville, MD; CBS, Centraalbureau voor Schimmelcultures, Utrecht; GAM, Julian H. Miller Mycological Herbarium, Athens, GA; IMI, CAB International, Egham; NY, New York Botanical Garden, Bronx, NY; OSC, Oregon State University Herbarium, Corvallis, OR; UAMH, University of Alberta Microfungus Collection and Herbarium, Edmonton, AB.

[b] Mistakenly initially identified as *S. kampalensis*; see Haugland & Heckman (1998).

[c] Epitype isolate fide Haugland *et al.* (2001).

characters, 549 are parsimony informative. All characters were used in the analyses. MP phylogenetic analysis of alignment 1 resulted in 48 equally parsimonious trees, differing slightly in the arrangement of the terminal taxa within the major lineages (length = 2948, CI = 0.384, RI = 0.636, RC = 0.244, HI = 0.616). Bayesian and MP phylogenetic analyses of alignment 1 both identified the same groups corresponding to the major orders of the *Sordariomycetes*. Fig. 1 shows one of three Bayesian trees generated from alignment 1, with posterior probabilities above and MP bootstrap supports below the branches for the major orders. Within the major orders, only minor branching differences were noted in the trees resulting from the two analyses. Isolates of *Stachybotrys* were placed within the *Hypocreales* with 70% MP bootstrap support and 100% posterior probability in the Bayesian analyses. In these analyses, *S. chartarum* was most closely allied with species of *Myrothecium*, another asexually reproducing genus. *Melanospora* and *Sphaerodes*, however, were not placed within the *Hypocreales* in any of the analyses contrary to previous hypotheses (Rehner & Samuels 1995, Zhang & Blackwell 2002) and were excluded from the hypocrealean dataset (alignment 2).

Alignment 2 consisted of nrSSU (1022 bp), nrLSU (914 bp), EF1-α (1014 bp), RPB1 (829 bp), and ATP6 (586 bp) sequences for 41 hypocrealean taxa and two phyllachoralean outgroup taxa for a total of 4365 total characters. Due to difficulties in aligning an intron-containing region in the RPB1 gene, 295 characters were excluded from that region in the analysis resulting in 4070 included characters of which 1119 are parsimony informative. MP phylogenetic analysis of alignment 2 resulted in two equally parsimonious trees (length = 7172, CI = 0.31, RI = 0.463, RC = 0.143, HI = 0.690). Fig. 2 shows one of three Bayesian trees generated for alignment 2, with posterior probabilities above, and MP bootstrap supports below, the family level branches. Supports for branches within recognized families are not shown and branches leading to family level groups that are present in the strict MP consensus tree are shown with thickened lines. In both analyses *Stachybotrys*, *Myrothecium*, *Peethambara spirostriata*, and *Didymostilbe echinofibrosa* form a monophyletic group with 100% MP bootstrap support and 100% Bayesian posterior probabilities.

DISCUSSION

The *Hypocreales* represent an order of ascomycetous fungi that are most successful and well-known in their asexually reproducing states, such as *Acremonium*, *Clonostachys*, *Cylindrocladium*, *Fusarium*, and *Trichoderma* (Rossman 2000). Because of their ability to degrade a wide range of substrates, hypocrealean fungi function as virulent plant pathogens, producers of powerful antibiotics, sources of potent mycotoxins, and effective agents for the biocontrol of invasive fungi, plants, or insects. Within the *Hypocreales*, the major families have been defined based on morphological characteristics and recognized as the *Bionectriaceae*, *Clavicipitaceae*, *Hypocreaceae*, *Nectriaceae*, and *Niessliaceae* (Rossman *et al.* 1999). When tested using DNA sequence data, these families continue to define the major known lineages of the *Hypocreales* (Okada, Takematsu & Takamura 1997, Rossman *et al.* 2001, Zhang & Blackwell 2002).

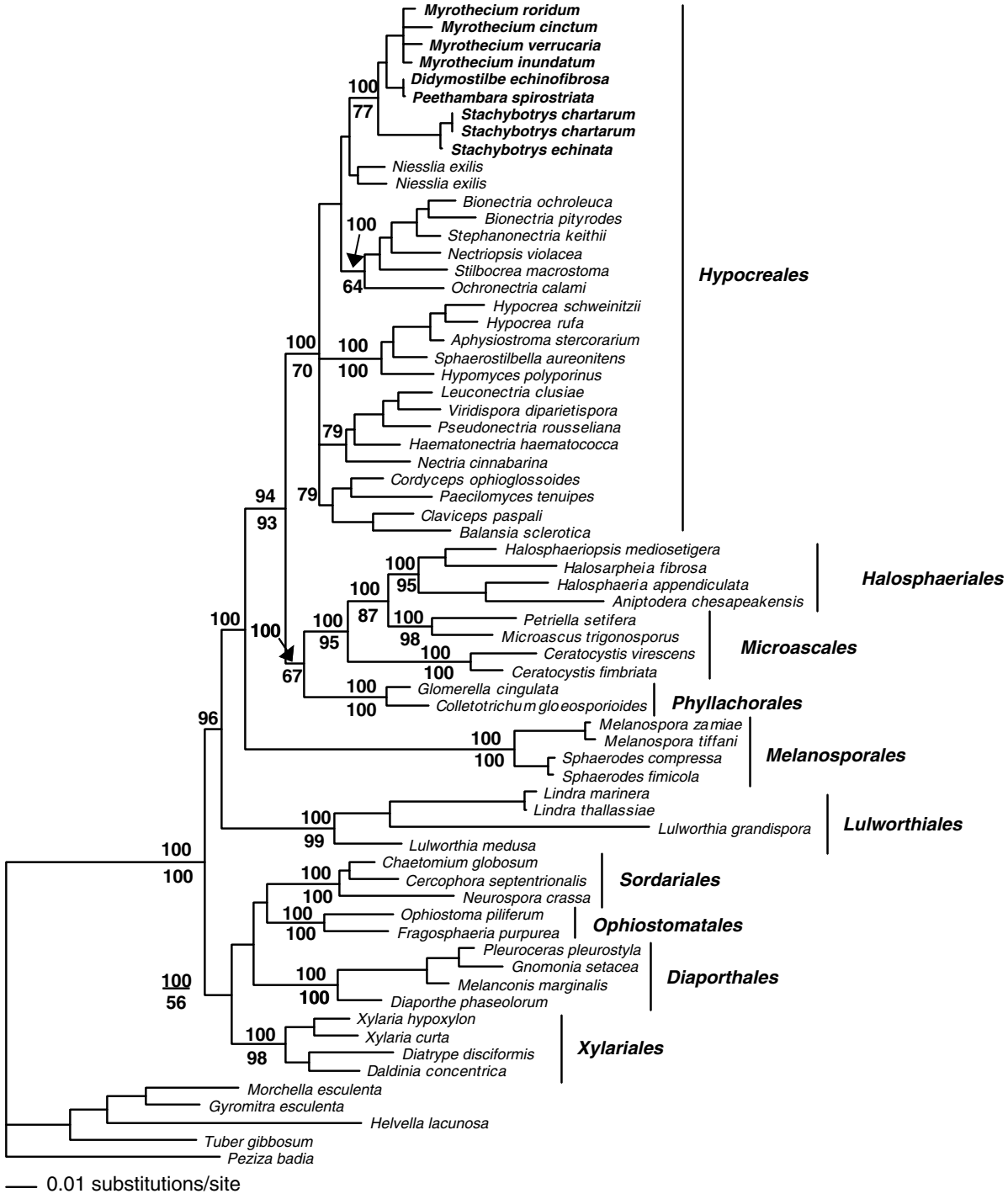

Fig. 1. Bayesian tree resulting from analysis of 1625 bp from nrSSU and nrLSU for the major orders of the *Sordariomycetes*. The numbers above the branches indicate pooled posterior probabilities obtained from three independent Bayesian analyses, each consisting of 500 000 Markov Chain Monte Carlo generations (GTR + Γ + I model), with a burn-in of 100 000 generations. Numbers below the branches indicate MP bootstrap support proportions from 1000 pseudoreplicates with ten random taxon addition replicates per pseudoreplicate for major lineages only.

The obscure family *Niessliaceae* was detailed based on morphological characteristics by Samuels & Barr (1997) and included in the *Hypocreales* (Rossman *et al.* 1999). However species of *Niesslia* have not been included in molecular phylogenetic analyses and placement within the *Hypocreales* has remained questionable (Kirk *et al.* 2001, Eriksson *et al.* 2004). All analyses performed in this study firmly support the placement of *N. exilis*, the type species of the genus *Niesslia*, in the *Hypocreales* with the Bayesian analysis of the 5-gene hypocrealean dataset (Fig. 2) suggesting a close relationship with the *Bionectriaceae*. The genus *Melanopsamma* based on the type species *M. pomiformis* (anamorph *Stachybotrys albipes*) was previously placed in the *Niessliaceae* (Samuels & Barr 1997, Rossman *et al.* 1999). The *Stachybotrys* lineage, including

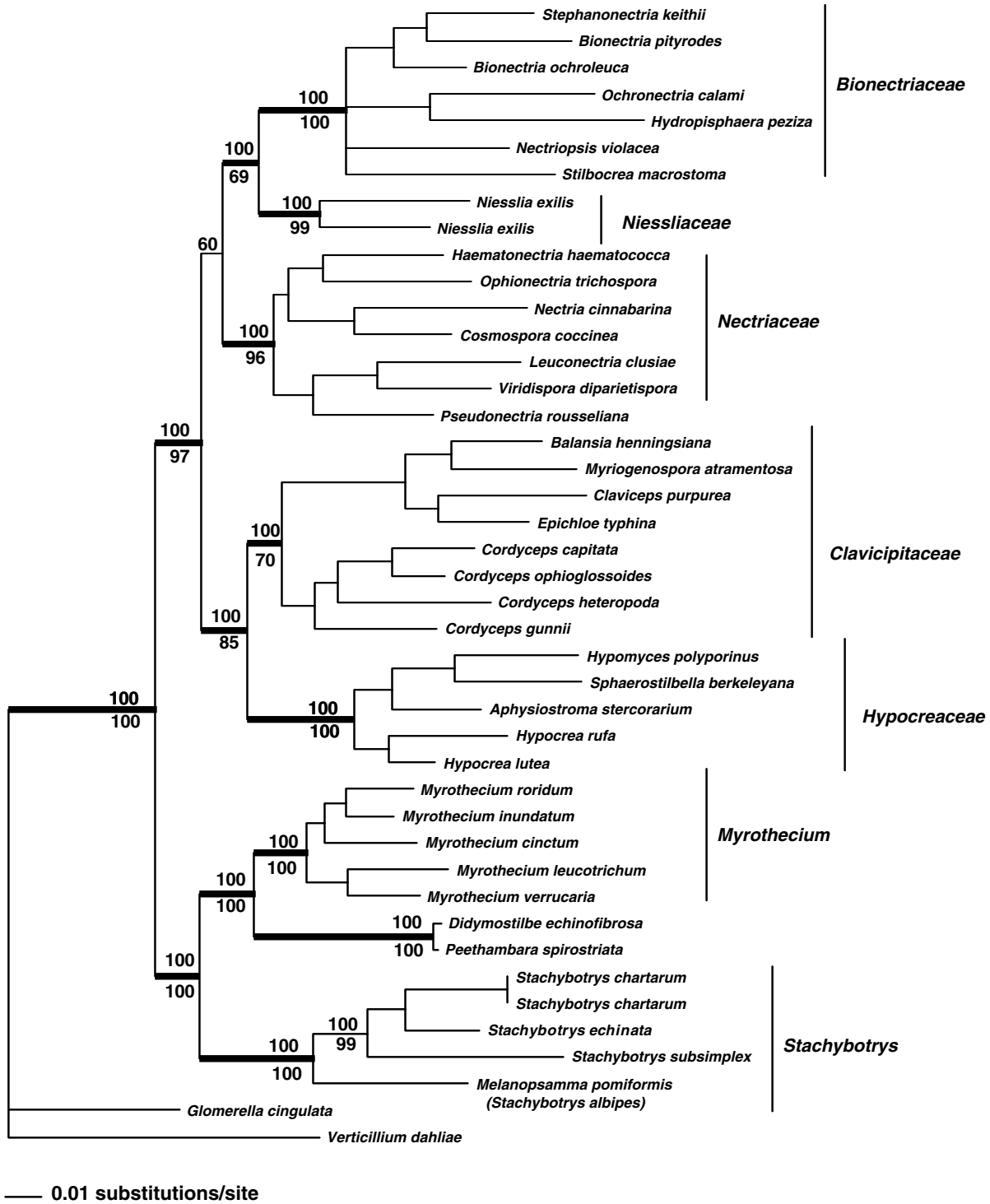

Fig. 2. Bayesian phylogeny for 41 hypocrealean and two phyllachoralean outgroup taxa based on 4070 bp from four nuclear (nrSSU, nrLSU, RPB1, EF1-α) and one mitochondrial (ATP6) genes. The numbers above the branches indicate pooled posterior probabilities obtained from three independent Bayesian anlyses, each consisting of 500 000 Markov Chain Monte Carlo generations (GTR + Γ + I model), with a burnin of 100 000 generations. Numbers below the branches indicate MP bootstrap support proportions from 1000 pseudoreplicates with 10 random taxon addition replicates per pseudoreplicate. Posterior probabilities and bootstrap supports are only indicated on the internal branches leading up to the family level groupings. Thickened lines indicate branches present in strict consensus MP tree leading up to the family level groupings, except for the *Stachybotrys*/*Myrothecium* lineage.

M. pomiformis, was not closely related to *N. exilis* in these analyses. Contrary to Samuels & Barr (1997), *Melanopsamma* is not a member of the *Niessliaceae*.

The closest relatives of the *Stachybotrys*/*Melanopsamma* lineage in these analyses were species of *Myrothecium*, another asexually reproducing genus,

and *Peethambara spirostriata*, an obscure sexually reproducing fungus, and its anamorph *Didymostilbe echinofibrosa* (syn. *Virgatospora echinofibrosa*). *P. spirostriata* was discovered only recently, and is known from just a few collections in tropical regions on decaying plant debris (Rossman 1983). Although its asexual state is more commonly reported, *D. echinofibrosa* also appears to be restricted to tropical areas (Ellis 1971 as *Virgatospora echinofibrosa*, Rossman 1983 under *Nectria spirostriata*, Seifert 1990). Both *Myrothecium* and *Peethambara* were previously thought to be allied with the *Bionectriaceae* (Rossman *et al*. 2001).

Most species of *Myrothecium* are cosmopolitan, associated with plant debris from both temperate and tropical regions (Farr *et al*. 2004), but species of *Myrothecium* have been reported as indoor air fungi (Levetin & Shaughnessy 1997). Despite their ecological success, no sexual state has been discovered for any species of *Myrothecium*. The genus *Myrothecium* is typified by *Myrothecium inundatum*, a species that occurs on fruit bodies of *Russula* (Tulloch 1972). The most commonly known species of *Myrothecium*, namely *M. cinctum*, *M. roridum*, and *M. verrucaria*, are known to degrade cellulose, and *M. verrucaria* has been reported as 'one of the most potent cellulose decomposers known' (Domsch *et al*. 1980). *M. verrucaria* and *M. roridum* are plant pathogens, with *M. verrucaria* being considered for use in the control of noxious weeds (Domsch *et al*. 1980, Abbas *et al*. 2001, 2002, Boyette, Walker & Abbas 2002).

The anamorphic genera *Stachybotrys*, *Myrothecium*, and *Didymostilbe* are united by morphological characteristics, such as their production of dark green conidia as well as characteristics of their conidiogenesis. The conidia tend to be elongated, fusiform, ellipsoid or cylindrical, smooth, striate, or roughened. In these genera, the conidiophores are penicillately arranged, bearing either 3–10 phialides directly, or 3–10 secondary conidiophores each of which produces a cluster of phialides; the phialides tend to be cylindrical. In *M. inundatum*, *M. leucotrichum* and *M. verrucaria*, the conidia are smooth. In *M. cinctum* and *D. echinofibrosa*, the conidia are distinctly striate. In most species of *Stachybotrys* the conidia are smooth or roughened to a greater or lesser extent.

Species of both *Stachybotrys* and *Myrothecium* produce macrocyclic trichothecenes including verrucarins and roridins. Species of *Myrothecium*, including *M. roridum* and *M. verrucaria*, produce a series of eight macrocyclic trichothecenes identical to those formed by the trichothecene-producing isolates of *S. chartarum* (Jarvis, Pavanasasivam & Bean 1985, Jarvis 1991). According to Andersen *et al*. (2002), *S. albipes*, the anamorph of *M. pomiformis*, did not produce any of the tested trichothecene metabolites. It is not known if *P. spirostriata*/*D. echinofibrosa* produces mycotoxins similar to those of *Stachybotrys* or *Myrothecium*.

In all analyses of the five-gene hypocrealean dataset, *Stachybotrys*/*Melanopsamma*, *Myrothecium*, and *Peethambara*/*Didymostilbe* form a strongly supported, previously undiscovered sister lineage to all other families currently accepted in the *Hypocreales*, the *Bionectriaceae*, *Clavicipitaceae*, *Hypocreaceae*, *Nectriaceae*, and *Niessliaceae*. Within this lineage, *Stachybotrys* and *Myrothecium* are well supported as monophyletic anamorphic genera and the relationship of *Melanopsamma pomiformis* to *Stachybotrys* is confirmed. The connection of *Melanopsamma* to *Stachybotrys* has been made only once by Booth (1957). Although this lineage seemingly comprises a newly discovered family within the *Hypocreales*, additional teleomorph isolates, including the type species of *Peethambara*, *P. sundara*, are required to fully justify formally describing the new family. Perithecia of *Peethambara* are large, yellow, collapsed cupulate, and quite distinct from the small, black, albeit collapsed and cupulate, perithecia of *Melanopsamma*.

This research shows that species of *Stachybotrys* and *Myrothecium* are closely related to each other and share morphological and molecular characteristics as well as the production of similar mycotoxins. Those seeking to resolve the problems posed by *S. chartarum* inhabiting moist cellulose-rich substrates in building interiors may also need to test for the presence of species of *Myrothecium* and, in tropical regions, the related *D. echinofibrosa*. Given the lack of systematic knowledge of tropical fungi and even of many saprophytic temperate fungi, one would expect to find additional members of this new lineage that occupy a similar niche on decaying, cellulose-rich plant material and that are capable of producing the same or similar toxic compounds. Such basic information is necessary for developing rapid identification tools (Haugland & Heckman 1998) for determining the presence of *S. chartarum* and related toxin-producing fungi in environmental samples.

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