

Reconstructing the Clavariaceae using nuclear large subunit rDNA sequences and a new genus segregated from *Clavaria*

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Abstract: Fungi that produce clavarioid fruit bodies have evolved independently many times in the Basidiomycota. The evolutionary significance of this morphology is difficult to interpret because the phylogenetic positions of many clavarioid fungi are still unknown. In this study we examined the phylogenetic diversity of the Clavariaceae *sensu lato* among Homobasidiomycetidae by adding partial nuclear large subunit ribosomal DNA sequences from clavarioid and corticioid fungi to a large euagaric dataset and analyzing them both together and separately. Our results indicate that the clavarioid morphology has evolved at least five times in the euagarics while the inclusion of type species enabled us to evaluate the taxonomic consequences of this polyphyletic distribution. Although the sampling available at present is incomplete, a qualitative assessment of our phylogenetic hypotheses indicates that the clavarioid habit might not be as evolutionarily labile as previously reported. We propose the new genus *Alloclavaria* to accommodate *Clavaria purpurea*, which is not related to *Clavaria* but is derived within the hymenochaetoid clade. The Physalacriaceae and Clavariaceae are redefined to reflect monophyletic groups, and the limits of *Clavaria*, *Clavulinopsis* and *Ramariopsis* should be reconsidered when additional data are available.

Key words: *Actiniceps*, agaric, *Chaetothyphula*, clamp connection, coral mushroom, *Dimorphocystis*, Eumycota, *Holocoryne*, Hymenomycetes, *Macrotyphula*, molecular systematics, morphological evolution, *Physalacria*, *Typhula*

INTRODUCTION

The elaborate structures of mushrooms that have evolved in the homobasidiomycete fungi are a source of fascination for professional and amateur mycologists alike. Among the diversity of fruit body forms, the club and coral (clavarioid) fungi are peculiar in that, unlike other complex fruit bodies to which they are

related, the hymenium is fully exposed to the environment. Ecologically clavarioid fungi are ubiquitous, distributed worldwide and have adopted a diverse array of nutritional modes, including saprotrophy, mutualism and parasitism. Molecular data have revealed that the clavarioid morphology is homoplastic (Pine et al 1999, Moncalvo et al 2002) and that there have been frequent transitions between clavarioid and either agaricoid (Hibbett 2004) or corticioid morphologies in the Homobasidiomycetidae (Hibbett and Binder 2002, Larsson et al 2004, Binder et al 2005). While these results suggest that the clavarioid morphology is evolutionarily labile, the incomplete sampling of clavarioid fungi and allied species reveals that such interpretations are probably premature. For instance these studies sampled only five of 18 genera of Clavariaceae *sensu lato* (Donk 1964) and only three species of *Clavaria* have been included in any single phylogenetic analysis (Pine et al 1999, Moncalvo et al 2002). Most critically, type species of many clavarioid genera have not been sampled. This incomplete sampling leaves gaps in our knowledge of the phylogenetic distribution of core groups, thus hindering our ability to discern the evolutionary significance of the clavarioid fruit body.

E.J.H. Corner completed the only global monograph of clavarioid fungi, based on nearly 50 y study including extensive fieldwork and examination of herbarium specimens from around the world (Corner 1950, 1970). In his original monograph Corner recognized 540 species in 27 clavarioid and “allied” genera in an artificial family, Clavariaceae (Aphylophorales), and segregated the genera into six “series” plus one unplaced genus, *Clavicornia* (Corner 1950). Corner’s treatments were based on extensive observation and documentation of hyphal characters and he emphasized many affinities to agaricoid, cantharelloid, hydroid, stereoid, corticioid and polyporoid fungi. His observations led him to postulate that the evolutionary ancestor of homobasidiomycetous mushrooms was clavarioid, from which all other fruit body forms were derived through transitional series (Corner 1970).

Donk (1964) attempted a more natural classification of the Aphylophorales by proposing 21 heterogeneous families with clavarioid, hydroid, stereoid and polyporoid genera. In a later supplement to his monograph Corner adopted a modified version of Donk’s classification, recognizing 38 clavarioid genera in 12 families (Corner 1970). In the Clavariaceae

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Donk (1964) recognized 18 genera while Corner recognized only four in his supplement: *Clavaria* Fr., *Clavulinopsis* v. Ov., *Ramariopsis* Donk and *Scytinopogon* Singer (Corner 1970). *Scytinopogon* was the most distinct of these genera, allied to *Thelephora* and defined by clavarioid fruit bodies with flattened branching, tough and pliant flesh, and echinulate or verrucose angularly elliptic spores (Corner 1950). The other three genera are distinguished by subtle and historically contentious morphological characters (Corner 1970, Petersen 1978) and their anatomical features often grade into one another.

Of the other clavarioid fungi treated in the Clavariaceae, the enigmatic genus *Physalacria* is of special interest because of its peculiar morphology. This unique "physalacrioid" fruit body is described generally as a balloon on a stick, where the pileus, consisting of an inflated swelling, sits atop a centrally attached stipe. Molecular data support the affinity of the physalacrioid species to marasmioid fungi (Moncalvo et al 2000, 2002; Binder et al 2005; Wilson and Desjardin 2005), but the position of the type species of *Physalacria*, *P. inflata*, remains unknown. Recently, an isolate of *P. inflata* has been placed in the core polyporoid clade with evidence from two nuclear and two mitochondrial ribosomal DNA sequences, suggesting that *Physalacria* is not monophyletic (Binder et al 2005). However the authors were skeptical of this placement and encouraged evidence from additional isolates to verify its position.

In this study we examined the phylogenetic diversity of the Clavariaceae by adding nuclear large subunit ribosomal DNA sequences from selected clavarioid and resupinate taxa to the euagaric dataset of Moncalvo et al (2002). We also examined the systematics of *Clavaria*, *Clavulinopsis* and *Ramariopsis* more closely and compared our results with Corner's and Petersen's alternate classifications (Corner 1970, Petersen 1978). We confirm that homoplasy is a prevalent feature in the Clavariaceae. Given the paucity of homobasidiomycete taxa sequences, it is still premature to evaluate trends of fruit body evolution. Nonetheless our results imply that clavarioid fungi might be derived mostly from other fruit body forms, contrary to recent evidence that they are evolutionarily labile (Hibbett 2004). In some cases it also appears that cyphelloid, flabellate and corticioid fungi may form intermediate or penultimate stages in the transition from agaricoid to clavarioid fruit bodies.

MATERIALS AND METHODS

Taxon sampling/DNA sequence acquisition.—Taxa in the Clavariaceae and Pterulaceae were selected with the systematic treatments of (Corner 1950, 1952a, 1952b,

1970) and (Petersen 1978, 1988) as guides (TABLE I). Isolates include recent and herbarium collections from the USA, Puerto Rico, Costa Rica, Singapore and New Zealand. New collections are deposited in the University of Minnesota Herbarium (MIN). A total of 22 new sequences from nine genera and 19 species were generated for this study. Additional taxa previously reported with affinities to Clavariaceae or Pterulaceae taxa (Larsson et al 2004, Binder et al 2005) also were included. Genomic DNA was extracted with the manufacturer's protocols using a modified CTAB/SDS extraction or one of two commercially available kits: QIAGEN DNeasy Tissue DNA Extraction Kit (Valencia, California) or Epicentre® MasterPure™ Yeast DNA Purification Kit (Madison, Wisconsin). The CTAB/SDS protocol was modified to include a 60 min incubation of the CTAB solution with 5 µL of proteinase K (20 mg/mL) at 65 C, and a 1–2 h treatment with RNase A (10 mg/mL) after extraction with a 25:24:1 solution of chloroform:phenol:isoamyl alcohol. DNA was precipitated with 100% isopropanol, washed with cold 70% ethanol, dried under vacuum, and resuspended in 50 µL Tris-EDTA buffer (1M). The first ca. 900–1400 bp of the 5' region of the nuclear large subunit ribosomal DNA (nrLSU) was amplified with published primers (Vilgalys and Hester 1990) in the combinations LROR (forward) and LR5 or LR7 (reverse). PCR amplification was achieved by diluting Sigma JumpStart™ Taq ReadyMix™ (St Louis, Missouri) 1:1 with a mixture of primers (final concentration 1 µM) and water, with or without betaine (final concentration 1M). Five microliters of DNA extract (diluted 1:1, 1:9, 1:99, or 1:999 in water) were added to 20 µL of the above reaction mixture and covered with one drop of sterile mineral oil. Amplification was completed with a touchdown program on a MJ Research PTC-100 thermal cycler (Bio-Rad Laboratories, Waltham, Massachusetts) with these parameters: (i) 94 C for 4 min, (ii) 94 C for 1 min, (iii) 60 C for 30 sec, (iv) 72 C for 1 min and 30 sec, (v) repeat steps 2–4 for 34 more cycles, stepping down annealing temperature by 1 C per cycle to 52 C, (vi) 94 C for 1 min, (vii) 52 C for 30 sec, (viii) 72 C for 10 min, (ix) hold indefinitely at 4 C. PCR products were viewed under UV light by running 3 µL of amplified reaction in a 1% agarose gel stained with ethidium bromide at 85V for ca. 1 h. Positive products were cleaned with QIAquick PCR Purification Kit (QIAGEN) following the manufacturer's instructions. Unidirectional dye-terminated sequencing for the forward and reverse reactions using the same primer combinations for PCR and the additional primers LR3R and LR16 (Vilgalys and Hester 1990) was conducted with an ABI PRISM™ 3700 DNA Analyzer (Foster City, California) at the BioMedical Genomics Center, University of Minnesota. Contiguous sequences were made in Sequencher 3.0 (Gene Codes Corp., Ann Arbor, Michigan) by overlapping the unidirectional reads.

Alignment and phylogenetic analyses.—Phylogenetic analyses were completed in two parts. The primary analyses included addition of new and published sequences to the dataset of (Moncalvo et al 2002), referred to here as the "Homobasidiomycetidae" dataset. The secondary analyses are composed of more extensive examinations of monophyletic

TABLE I. Taxon, collection data, voucher, GenBank and reference information for sequences used in this study. This list does not include taxa originally included in Moncalvo et al (2002) or 55 sequences relevant to the Pterulaceae that will be published separately.

Taxon	Location/Date	Habitat	Voucher ID	GenBank ID	References
<i>Actiniceps laevis</i> (Corner) Boedijn	Singapore, Upper Seletar Reservoir, 16 Mar 2004	In forest on fallen leaf of <i>Syzygium cerena</i>	DJM1350	DQ284917	this study
<i>Athelia arachnoidea</i>			815	AF518601	Binder et al 2005
<i>Athelia arachnoidea</i>			GEL2529.1	AJ406484	Binder et al 2005
<i>Athelia epiphylla</i>			HHB-8546-sp	AY293168	Binder et al 2005
<i>Athelia fibulata</i>			GEL5292	AJ406485	Binder et al 2005
“ <i>Bulbillomyces</i> <i>farinosus</i> ”			FO24378	AJ406578	Binder et al 2005
<i>Chaetothyphula</i> <i>hyalina</i> (Jungh.) Corner	Singapore, Botanic Gardens’ Rainforest, 12 Mar 2004	On lamina of decaying fallen frond of <i>Ptychosperma</i> sp.	DJM1348	DQ284912	this study
<i>Chaetothyphula</i> sp.	Puerto Rico, Carribean National Forest, Caimitilla Trail, 26 Nov 1996	On <i>Cecropia</i> bract and petiole on ground	DJM1031	DQ284910	this study
<i>Chaetothyphula</i> sp.	Costa Rica, San Jose Province, near Santa Maria de Dota, Jardín de Bienvenidos, 5 Jun 2004	On decaying monocotylendous stem	BDCR0419-2c	DQ284896	this study
<i>Clavaria argillacea</i>			Elj-98	AY463395	Larsson et al 2004
<i>Clavaria fumosa</i>			KGN98	AY586646	Larsson et al 2004
<i>Clavaria purpurea</i> Fr.	Oregon Dunes National Recreation Area, Waxmyrtle Trail, Lane Co., 14 Jan 2002	On sandy soil in pine needle duff under <i>Pinus contorta</i>	DJM1317	DQ284899	this study
<i>Clavaria purpurea</i> Fr.	Minnesota, St. Louis Co., off Reservoir Forest Management Rd. (near Co. Rd. 44), 9 Oct 2005	On ground among moss near <i>Abies</i> <i>balsamea</i> in mixed woods	BD299	DQ284900	this study
<i>Clavaria purpurea</i> Fr.	Oregon Dunes National Recreation Area, Stagecoach Trail, Lane Co., 2 Jan 2003	On moss covered bank under evergreen huckleberry, salal, <i>Picea sitchensis</i> and waxmyrtle	DJM1334	DQ284898	this study
<i>Clavaria redoleo-alli</i> R.H. Petersen	New Zealand, Waipoua Kauri Forest, Yakas Track, Hokianga Co., 350 m elev., 15 May 1997	On soil and plant debris	DJM1079	DQ284906	this study
<i>Clavaria vermicularis</i> Fr.	Minnesota, Nerstrand Big Woods State Park, Fox Trail, Rice Co., 12 Aug 2000	On humus in mixed deciduous forest	DJM1262	DQ284907	this study
<i>Clavaria zollingeri</i>			AFTOL563	AY639882	AFToL ^a
<i>Clavulicium delectabile</i>			KHL11147	AY586688	Larsson et al 2004
<i>Clavulina cristata</i> (Holmsk.) J. Schröt	Wisconsin, chestnut forest near West Salem, 21 Sep 2001	On ground under <i>Castanea dentata</i> with spruce and pine nearby	DJM1297	DQ284901	this study
<i>Clavulinopsis</i> / <i>Ramariopsis helvola</i>			RD990908	AY586647	Larsson et al 2004
<i>Clavulinopsis</i> / <i>Ramariopsis laeticolor</i>			AFTOL984	AY745693	AFToL ^a
<i>Clavulinopsis</i> / <i>Clavaria sulcata</i> (Overeem) R.H. Petersen	New Zealand, Bay of Plenty, Katikati, Aongatete Lodge, 8 May 2003	On ground in forest	PDD78241	DQ284904	this study

TABLE I. Continued

Taxon	Location/Date	Habitat	Voucher ID	GenBank ID	References
<i>Coronicium alboglaucum</i>			GEL5058	AJ406487	Langer unpubl
<i>Henningsomyces candidus</i>			GEL4482	AJ406553	Binder et al 2005
<i>Hyphoderma praetermissum</i>			GEL2182	AY700185	AFToL ^a
<i>Kavinia alboviridis</i>			EL16-98	AY463434	Larsson et al 2004
<i>Kavinia himantia</i>			FP-101479	AY293190	Binder et al 2005
<i>Kavinia himantia</i>			LL-98	AY586682	Larsson et al 2004
<i>Kavinia himantia</i> (Schwein.) J. Erikss.	New Zealand, Waipoua Kauri Forest, Kauri Rikers Trail, Hokianga Co., 60 m elev., 14 May 1997	On vertical twigs and attached fallen fern pinnae	DJM1070	DQ284916	this study
<i>Kavinia</i> sp.			FO25092	AJ406489	Langer unpubl
<i>Lachnocladium</i> sp.			KHL10556	AF506461	Larsson et al 2004
<i>Lentaria albovinacea</i>			GEL5388	AJ406552	Binder et al 2005
<i>Macrotyphula defibulata</i> R.H. Petersen	New Zealand, Rimutake State Forest, 15 km southwest of Wellington, Catchpool Valley, Orongorongo Track, 80–100 m elev., 8 May 1997	On leaves, often of <i>Knightia</i> sp., and twigs on ground in moist broadleaf forest with <i>Nothofagus</i> , tree ferns, podocarps and palms	DJM1056	DQ284897	this study
<i>Macrotyphula juncea</i> (Alb. & Schwein.) Berthier	Puerto Rico, Carribbean National Forest, Caimitilla Trail, 26 Nov 1996	On little decayed leaves on ground in palm and <i>Cecropia</i> forest	DJM1032	DQ284911	this study
<i>Macrotyphula juncea</i> (Alb. & Schwein.) Berthier	Costa Rica, La Amistad International Park, Stanford Univ. Biological Station, Las Alturas, Trail C, ca. 1580 m elev., 26 Jul 1995	On leaf litter in woods	DJM975	DQ284909	this study
<i>Membranomyces delectabilis</i> (= <i>Clavulicium delectabile</i>)			KHL11147	AY586688	Larsson et al 2004
<i>Mucronella calva</i>			GEL4458	AJ406588	Langer unpubl
<i>Mucronella fusiformis</i> (Kauffman) K.A. Harrison	Oregon, Siuslaw National Forest, Cape Perpetua Special Interest Area, Lincoln Co., 4 Jan 2002	On well rotted, very wet, fallen stump of ancient tree	DJM1309	DQ284905	this study
<i>Physalacria corticola</i> Corner	Singapore, Botanic Gardens' Rainforest, 12 Mar 2004	On fallen twig under <i>Ptychosperma</i> sp.	DJM1349	DQ284913	this study
" <i>Physalacria inflata</i> "			HHB-13443-sp	AY293201	Binder et al 2005
<i>Physalacria inflata</i> (Schwein. ex Fr.) Peck	Minnesota, Nerstrand Big Woods State Park, Basswood Loop, Rice Co., 9 Sep 2005	On cut end of large, decaying hardwood log in mixed harwood forest	BD347	DQ284915	this study
<i>Physalacria maiipoensis</i>			2373Inderbit zin	AF426959	Hibbett and Binder 2001

TABLE I. Continued

Taxon	Location/Date	Habitat	Voucher ID	GenBank ID	References
<i>Physalacria orinocensis</i> Pat. & Gaillard	Louisiana, Burden Plantation, East Baton Rouge Parish, 29 Nov 1996	On twig on ground in bottomland hardwoods	DJM1035	DQ284914	this study
<i>Physalacria</i> sp.			GEL5189	AJ406593	Langer unpubl
<i>Pistillaria micans</i> (Pers.) Fr.	California, University of California, Berkeley, 9 Apr 1967	On fruits of <i>Pittosporum</i> sp.	M504	DQ284908	this study
<i>Ramariopsis kunzei</i> (Fr.) Corner	Minnesota, Nerstrand Big Woods State Park, Basswood Loop, Rice Co., 9 Sep 2005	On ground in mixed hardwood forest, mostly <i>Acer</i> spp. nearby	BD346	DQ284902	this study
<i>Ramariopsis</i> Taxon no. 4 (Petersen 1988)	New Zealand, Kaitoke Regional Park, north of Hutt, trails near Forks Carpark, 7 May 1997	On humus in broadleaf and podocarp forest with rimu, rata and <i>Nothofagus</i>	DJM1049	DQ284903	this study
<i>Typhula phacorrhiza</i>			DSH96-059	AF393079	Binder and Hibbett 2002
<i>Typhula phacorrhiza</i>			EL43-99	AY586724	Larsson et al 2004

^aAssembling the Fungal Tree of Life (<http://ocid.nacse.org/research/aftol>)

clades identified from the initial analysis of the Homobasidiomycetidae dataset. All analyses were conducted either on a personal Macintosh G3 iBook or a SunFire 6800 running UNIX in the Computational Genetics Laboratory and/or a compute node of 733 MHz Intel Pentium III processors running RedHat Enterprise Linux 3 at the Netfinity Linux Cluster, Supercomputing Institute, University of Minnesota. All data matrices and trees have been submitted to TreeBASE (SN2545).

Primary matrix—Homobasidiomycetidae. Partial sequences from the 5' end of the nrLSU from 33 clavarioid fungi (20 new, 13 from GenBank), 23 corticioid (including hydnooid-resupinate) fungi (three new, 20 from GenBank), 47 pteruloid fungi (including *Apterostigma*-ant cultivars; 11 new, 36 from GenBank) and one agaric (from GenBank) were manually aligned to the euagaric dataset (877 sequences) of Moncalvo et al (2002) with the data editor window of PAUP*v4b10 (Swofford 1998). A total of 49 sequences relevant to the Clavariaceae are reported in this study (TABLE I) and the other 55 will be reported in a separate publication on the Pterulaceae. Some sequences were truncated at the beginning and/or end to fit the alignment. Alignments of positions 647–704 and after position 1000 were not optimized because they were excluded from all analyses (Moncalvo et al 2002). The maximum parsimony (MP) criterion was used to select among a set of trees generated by 10 independent heuristic searches with the parsimony ratchet (Nixon 1999) implemented in PAUPRat (Sikes and Lewis 2001). Each "ratchet" was executed for 1000 iterations with 15% of characters weighted. An equally weighted parsimony bootstrap tree was constructed with 100 replicates of heuristic searches with random sequence addition, TBR branch-swapping, MAXTREES set to 10, holding a single tree at each step, and MULTTREES option in effect.

Secondary matrices. Topologies and bootstrap values within terminal clades might contain artifacts from the broad taxonomic sampling and the suboptimal methods used to analyze this large dataset. Therefore we compared the results of the 50% majority rule consensus tree of the 137 equally parsimonious trees with the MP bootstrap tree to identify clades of clavarioid fungi and their closest relatives to analyze separately with MP, maximum likelihood (ML) and Bayesian (BA) methods. Five datasets were analyzed separately: "Hymenochaetoid clade," "Macrotyphula clade," "Chaetothyphula clade," "Physalacriaceae clade" and "Clavaria clade." For each dataset sequence alignments were optimized manually and the uneven ends were truncated further before all analyses. Except for a six base-pair region in the *Macrotyphula* clade dataset and 87 positions within the *Clavaria* clade dataset, there were no regions of ambiguous alignment after optimization. All ambiguously aligned positions, parsimony uninformative characters and ends were removed before the MP analyses (ML and BA included parsimony-uninformative characters). Exhaustive or branch-and-bound searches (for datasets with $\leq 12 >$ taxa, respectively) were completed for MP with PAUP*v4b10, and metropolis-coupled Markov chain Monte Carlo simulations for BA with MrBayes v3.1.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Heuristic searches under the ML criterion began with estimating model parameters on the most parsimonious trees (or one of a set of equally parsimonious trees) from the MP search, then searching with TBR branch-swapping on the MP tree with the new estimated parameter values. Model parameters were re-estimated on the new ML tree, and a second heuristic search with the new parameters and TBR branch swapping followed. This process was repeated iteratively until the parameter estimates and negative log likelihood values stabilized. For the ML and BA analyses, the model of

molecular evolution was selected under the Bayesian information criterion (BIC) with Modeltest v3.7 (Posada and Crandall 1998). The BIC is considered to have properties that are advantageous over the hierarchical likelihood ratio test for determining appropriate models of evolution (Posada and Buckley 2004) and was used to select all models for likelihood-based analyses in this study. Nonparametric bootstrapping with 1000 heuristic bootstrap pseudoreplicates, each with 100 random sequence addition replicates and TBR branch swapping, was used to assess branch support in the MP and ML analyses. ML bootstrap searches were conducted with model parameter values estimated in the iterative PAUP* searches. Bayesian analyses were executed with the default (uniform) prior parameters in MrBayes v3.1. Four parallel Markov chains were run for 1 000 000 generations, sampling every 100th tree, with two independent runs per analysis. Chain stationarity was evaluated with summary statistics calculated with the "sump" command (e.g. sufficient swapping among chains, standard deviation of split frequencies <0.01, potential scale reduction factors near 1) and by visually inspecting the generation vs. log probability of observing the data plots. For all BA analyses the first 2500 trees (25%) from each run were discarded as "burn-in," and a majority rule consensus tree of the last 15 002 trees was generated automatically in MrBayes using the "sumt" command.

RESULTS

Primary matrix.—Homobasidiomycetidae.—Dataset and alignment. After 123 redundant sequences were removed (Moncalvo et al 2002), the dataset consisted of 862 taxa. Of 1579 aligned positions, 801 were considered ambiguous and removed before all analyses, leaving 778 characters: 447 variable characters were parsimony informative, 139 variable characters were parsimony uninformative and 192 characters were constant.

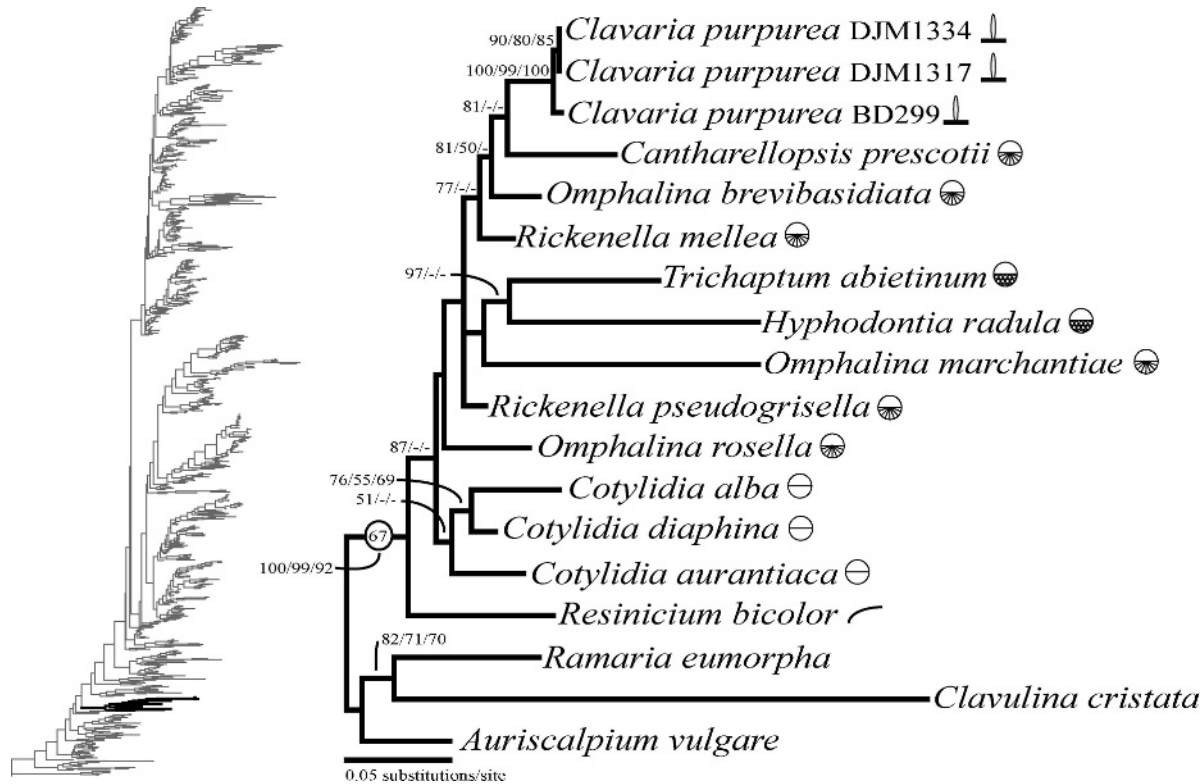
Phylogenetic analyses. Maximum parsimony analysis using the parsimony ratchet with uninformative and constant characters removed yielded a set of 137 shortest trees of equal length (L = 11956, CI = 0.082, RI = 0.678, RCI = 0.055). A single ratchet terminated prematurely at iteration 940 because the computer timed out. The final 60 iterations of this ratchet were considered to be an insignificant addition to the pool of 9940 trees and so it was not repeated. A 50% majority rule consensus of the 137 equally parsimonious trees (not shown) recovered the eight major clades of homobasidiomycetes (Hibbett and Thorn 2000) plus the "athelioid" clade (Larsson et al 2004, Binder et al 2005) and was largely congruent with the results of Moncalvo et al (2002). The MP bootstrap tree recovered seven of the major clades but placed the bolete clade (bootstrap 41%) in an unsupported position within the euagarics clade, next to the schizophyllum clade (bootstrap 1%). However this

result is consistent with the unstable basal relationships of the euagarics clade reported by Moncalvo et al (2002).

*Distribution of clavarioid fungi and secondary matrices.—*The distribution of pterulaceous fungi and their allied taxa will be reported in a separate publication. After each heading below, tree statistics for the analyses conducted on the secondary matrices are reported as follows: number of equally parsimonious trees, tree length, consistency index, retention index, rescaled consistency index; model of evolution and negative log likelihood score of the tree that maximized the likelihood of the data. Throughout this paper monophyletic clades are identified with the notation/cladename. Where appropriate references are made to the clades identified by Moncalvo et al (2002). Branch support from the primary euagarics MP bootstrap analysis is indicated with the notation (euMP), and support generated in the secondary analyses is identified as percentages, using this notation: BA posterior probability/ML bootstrap/MP bootstrap.

Hymenochaetoid clade. Trees = 7, L = 495, CI = .461, RI = .438, RCI = .202; TrN+Γ+I, -lnL 4148.32350. The Hymenochaetoid clade (FIG. 1) including three isolates of *Clavaria purpurea* (two from Oregon, USA, DJM1317 and DJM1334, and one from Minnesota, USA, BD299) was recovered in all 137 equally parsimonious trees and in the MP bootstrap tree with moderate support (euMP, 67%). The secondary matrix (18 taxa) was composed of all hymenochaetoid taxa from Moncalvo et al (2002) plus *C. purpurea* and rooted with one member each of the /gomphoid-phalloid (*Ramaria eumorpha*), /cantharelloid (*Clavulina cristata*) and /russuloid (*Auriscalpium vulgare*) clades. Of 896 aligned positions, seven were ambiguous and removed before all analyses: 152 characters were parsimony informative, 138 variable characters were parsimony uninformative and 599 were constant. No incongruence between the MP and ML trees was observed for moderately and strongly supported branches. The monophyly of the /hymenochaetoid clade received strong support (100/99/92) as did all three isolates of *Clavaria purpurea* (100/99/100).

Macrotyphula clade. Tree = 1, L = 85, CI = .694, RI = .735, RCI = .510; TrN+Γ+I, -lnL 1980.72522. A novel clade /macrotyphula (FIG. 2) is recovered with strong support (euMP, 85%) containing the clavarioid species *Macrotyphula juncea* (two isolates), *M. defibulata*, *M. fistulosa*, *Typhula phacorrhiza* (three isolates) and "*Bulbillomyces farinosus*." All of the 137 equally parsimonious trees recovered /phyllotopsis (clade 52) as the sister group, which received moderate bootstrap



FIGS. 1–5. In the phylograms numbers in circles are maximum parsimony nonparametric bootstrap values from the Homobasidiomycetidae dataset. Numbers labeling branches are Bayesian posterior probability/maximum likelihood bootstrap/maximum parsimony bootstrap. 1. A phylogram of one of 137 equally parsimonious trees of the Homobasidiomycetidae dataset is shown on the left with the branch leading to the “Hymenochaetoid clade” in bold. On the right is a maximum likelihood phylogram ($-\ln L$ 4148.3235) of the Hymenochaetoid clade after iterative TBR-swapping and re-estimation of model parameters using TrN+ Γ +I model. Rooted with outgroup *Ramaria eumorpha*, *Clavulina cristata* and *Auriscalpium vulgare*. Symbols after terminal taxa indicate fruit body morphology: clavarioid (\downarrow), agaricoid (\oplus), polyporoid (\ominus), corticioid (\curvearrowright) and flabellate ($\omin�$).

support (euMP, 61%). The secondary matrix consisted of /macrotyphula with /phyllotopsis as outgroup (11 taxa). Of 870 aligned positions 52 characters were parsimony informative, 57 variable characters were parsimony uninformative and 761 were constant. The MP and ML trees were topologically identical. Branch support from MP, ML and BA all suggest that *Macrotyphula* and *Typhula* are paraphyletic (FIG. 2): One branch leads to *Macrotyphula fistulosa* and “*Bulbillomyces farinosus*” (100/98/97), while the other branch leads to two isolates of *Macrotyphula juncea* and one isolate of *M. defibulata* (99/73/87). *Typhula phacorrhiza* is paraphyletic with one isolate (AF393079) strongly supported as a sister lineage to *Macrotyphula defibulata* and *M. juncea* (100/94/95) and two other isolates (AY586724, DAOM195241) strongly supported as monophyletic (100/100/100) and weakly supported as the sister lineage to all other species of /macrotyphula (65/54/84). A sister relationship of /macrotyphula is recovered with moderate support (euMP, 61%) to the cyphelloid *Henning-*

somyces candidus and the agarics *Phyllotopsis nidulans* and *Pleurocybella porrigens*. This is an amendment to the /phyllotopsis clade 52 of Moncalvo et al (2002).

Chaetotyphula clade. Tree = 1, L = 144, CI = 0.611, RI = 0.646, RCI = 0.395; TrN+I, $-\ln L$ 2202.40378. A novel monophyletic clade /chaetotyphula (FIG. 3) in the euagarics is recovered with strong support (euMP, 73%) containing *Chaetotyphula* spp. (Clavariaceae) and *Actiniceps laevis* (Pterulaceae). All 137 equally parsimonious trees recovered the /hemimycena clade (clade 23) in a sister relationship with /chaetotyphula, although bootstrap support was only moderate (euMP, 46%). In 72% of the equally parsimonious trees, the /adonis clade (clade 26) with *Pleurotopsis longinqua* was recovered as the sister group to /chaetotyphula and /hemimycena, but bootstrap support was weak (euMP, 7%). The secondary matrix consisted of the /chaetotyphula and /hemimycena clades as ingroups and the /adonis with *Pleurotopsis longinqua* as outgroup (11 taxa). After optimization of the alignment, there were 863 homologous positions:

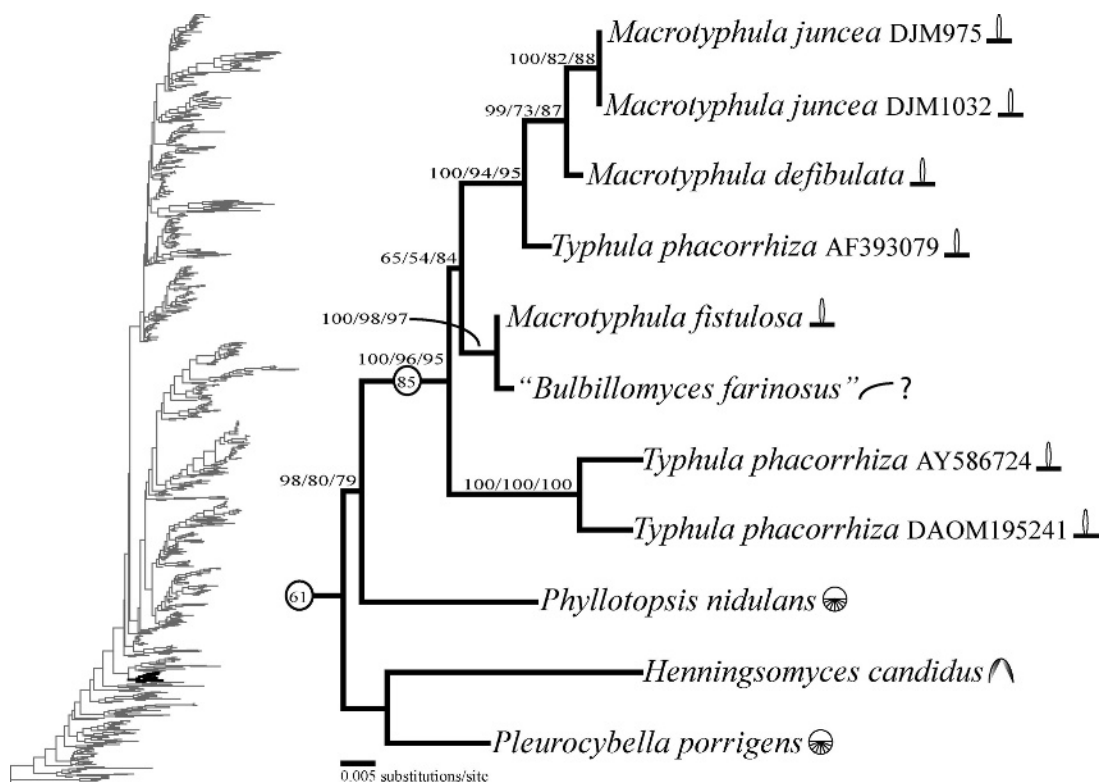


FIG. 2. A phylogram of one of 137 equally parsimonious trees of the Homobasidiomycetidae dataset is shown on the left with the branch leading to the “*Macrotyphula* clade” in bold. On the right is a maximum likelihood phylogram ($-\ln L$ 1980.72522) of the *Macrotyphula* clade after iterative TBR-swapping and re-estimation of model parameters using TrN+ Γ +I model. Rooted with the outgroup *Henningsomyces candidus* and *Pleurocybella porrigens*. Symbols after terminal taxa indicate fruit body morphology: clavioid (\perp), agaricoid (\ominus), corticioid (\curvearrowright) and cyphelloid (\wedge); ? indicates fruit body type is tentative based on dubious taxon identification.

72 were parsimony-informative, 45 variable characters were parsimony uninformative and 746 characters were constant. The MP and ML trees differed in the position of *Calyptrella capula*, where the MP tree recovered it at the base of the /chaetotyphula and /adonis clades and ML recovered it at the base of the /chaetotyphula clade, although neither of the methods provide significant branch support for either relationship. The /chaetotyphula clade was recovered in all analyses with strong support (100/83/79). The ingroup is also recovered with strong support (99/94/96), but the basal position of *Hemimycena delicatella* is barely not significant in the ML bootstrap and BA and is not supported by MP (91/67/-).

Physalacriaceae clade. Trees = 2, L = 253, CI = .573, RI = .714, RCI = .409; TrN+ Γ +I, $-\ln L$ 2985.41265. *Physalacria* is polyphyletic with one isolate (*Physalacria* sp.; GEL5189) closely related to the euagarics *Marasmius capillaris* and *M. rotula* in the /marasmioid clade 21 (euMP, 100%; not shown), one isolate of the type species (*P. inflata*; HHB-13443-sp) related to *Irpex lacteus* and *Trametes suaveolens* in the /polyporaceae (euMP, 100%; not shown), and five

other isolates including one isolate of the type species (*P. inflata*, BD347; *P. aff. orinocensis*, *P. orinocensis*, DJM1035; *P. corticola*; *P. maipoensis*) forming a monophyletic clade with the euagaric *Gloiocephala spathularia* (euMP, 85%; /physalacria clade 11, Moncalvo et al 2002; FIG. 4). A clade containing *Rhodotus palmatus*, *Xerula* spp., *Oudemansiella canarii*, *Flammulina velutipes*, *Strobilurus trullisatus*, *Rhizomarasmius pyrrophophalus*, *Cyptotrama asprata*, *Gloiocephala* spp., *Armillaria* spp. and /physalacria was recovered with strong support (euMP, 85%). This is the /physalacriaceae (clade 9) according to Moncalvo et al (2002). In the /physalacriaceae, a monophyletic /armillaria (clade 16; euMP, 69%) received moderate to strong support (euMP, 69%) as the sister group to the rest of the /physalacriaceae. Thus the secondary matrix consisted of the /physalacriaceae with /armillaria as outgroup (19 taxa). After optimizing the alignment by hand, there were 902 homologous positions: 112 were parsimony-informative, 73 variable characters were parsimony-uninformative and 717 were constant. The two MP trees differed in the resolution of *Physalacria maipoensis*, *P. orinocensis* and *P. aff. orinocensis*. The

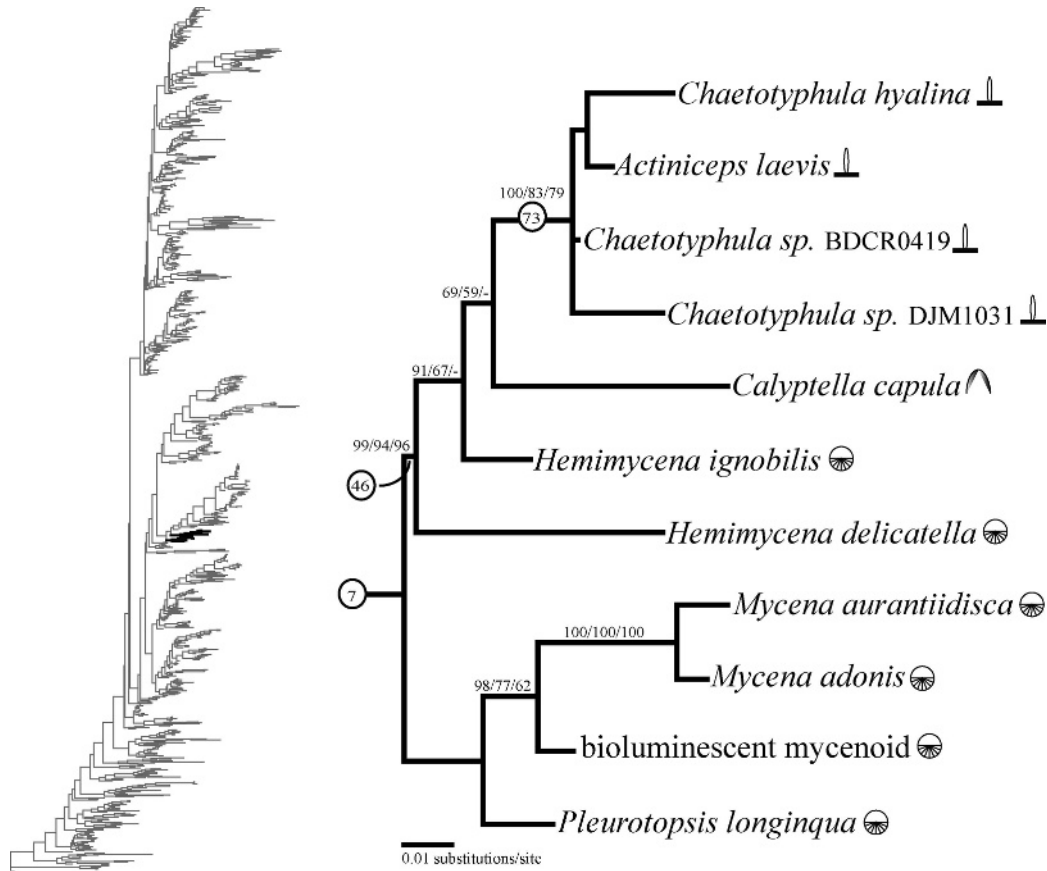


FIG. 3. A phylogram of one of 137 equally parsimonious trees of the Homobasidiomycetidae dataset is shown on the left with the branch leading to the “*Chaetothyphula* clade” in bold. On the right is a maximum likelihood phylogram ($-\ln L$ 2202.40378) of the *Chaetothyphula* clade after iterative TBR-swapping and re-estimation of model parameters using TrN+I model. Rooted with outgroup *Mycena aurantiidisca*, *Mycena adonis*, bioluminescent mycenoid JMC.R.32 and *Pleurotopsis longinqua*. Symbols after terminal taxa indicate fruit body morphology: claviroid (\perp), agaricoid (\oplus) and cyphelloid (\wedge).

ML tree was identical in topology to one of the two MP trees. The monophyly of the /physalacriaceae with the exclusion of /armillaria, and the monophyly of the /physalacria clade including one isolate of the type species (*P. inflata*, BD347), both received strong support (100/100/100 and 100/99/99, respectively). However *Physalacria* appears to be paraphyletic with *P. inflata* sharing a most recent common ancestor with *Gloiocephala spathularia* (100/100/100) and all other *Physalacria* species forming a monophyletic sister group (98/88/98). Other relationships within /physalacriaceae received moderate to strong support, but are not the focus of this study.

Clavaria clade. Tree = 1, L = 456, CI = 0.515, RI = 0.647, RCI = 0.333; TrN+I, $-\ln L$ 3707.2144. A core clade (FIG. 5) that we identify as Clavariaceae *sensu stricto*, including the type species of both *Clavaria* and *Ramariopsis*, was recovered with strong support (euMP, 94%). Although Moncalvo et al (2002) report the agaric clade /tricholomopsis (*Tricholomopsis rutilans*, *Marasmius rhyssophyllus*, *Collybia aurea*) as

possibly the sister lineage to *Clavaria fusiformis* (= *Ramariopsis* fide Petersen 1978), this relationship did not receive strong support, and their analysis included only this single representative of *Clavaria*. With seven species of *Clavaria* and four of *Ramariopsis*, our analysis did not recover this relationship in any of the MP trees or the MP bootstrap tree. However we did recover a sister relationship of Clavariaceae *s. str.* to *Mucronella* spp. and *Phlebia tristis* in 72% of the set of 137 equally parsimonious trees. In addition a sister relationship of the Clavariaceae *s. str.* and *Mucronella* to the /gymnopiloid (clade 97; *Gymnopilus* spp., *Hebelomina neerlandica* and *Galerina paludosa*) also was recovered in 72% of the MP trees. Therefore we aligned the Clavariaceae *s. str.* with *Mucronella*, /gymnopiloid, and /phylloopsis and used /bolete taxa as outgroup to assess the relationships of the four ingroup clades to each other. Using MP, ML and BA methods the Clavariaceae *s. str.* was unambiguously placed with *Mucronella* and the /gymnopiloid clade was recovered as the sister group of Clavariaceae *s. str.*

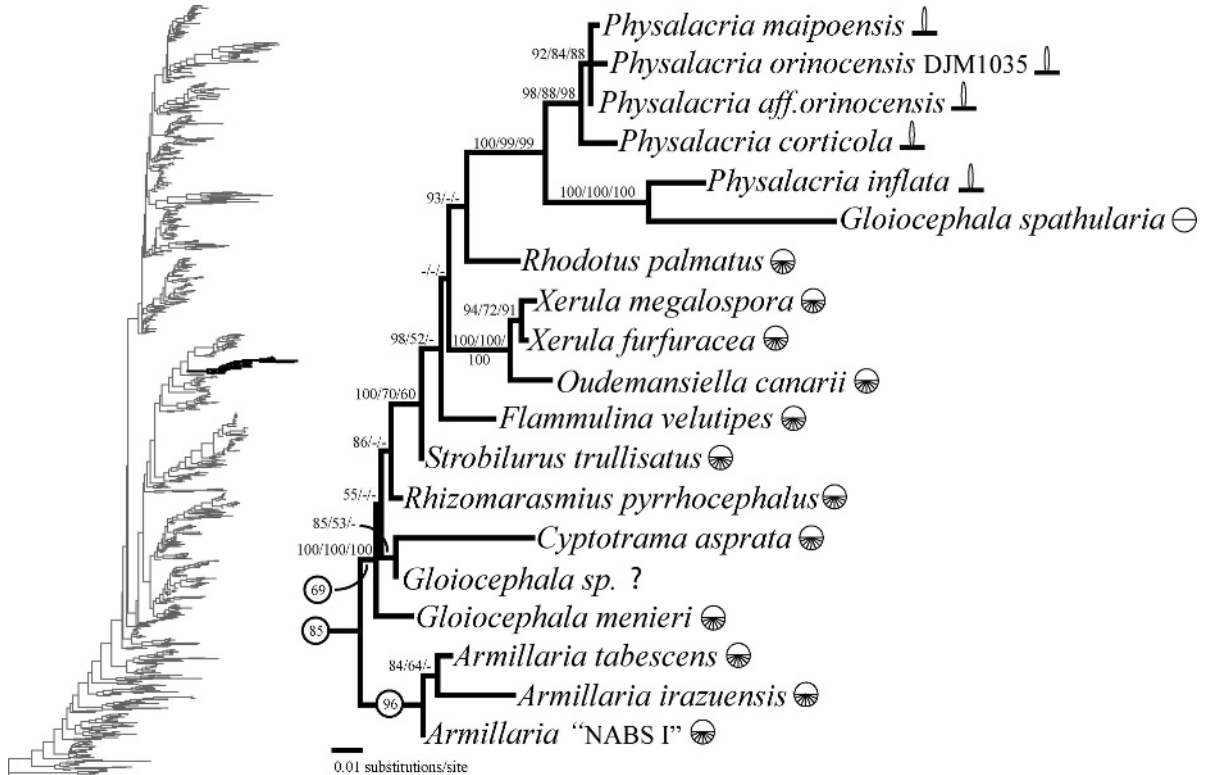


FIG. 4. A phylogram of one of 137 equally parsimonious trees of the Homobasidiomycetidae dataset is shown on the left with the branch leading to the “Physalacriaceae clade” in bold. On the right is a maximum likelihood phylogram ($-\ln L$ 2985.41265) of the Physalacriaceae clade after iterative TBR-swapping and re-estimation of model parameters using TrN+ Γ +I model. Rooted with the outgroup *Armillaria tabescens*, *Armillaria irazuensis* and *Armillaria* ‘NABS I’. Symbols after terminal taxa indicate fruit body morphology: clavarioid (⌋), agaricoid (☉) and spathulate (⊖); ? indicates unknown fruitbody type.

plus *Mucronella* with strong support in all analyses (not shown). Therefore for the *Clavaria* clade dataset we chose the /gymnopiloid clade as outgroup and Clavariaceae s. str. and *Mucronella* as ingroup. After removing the uneven ends (531 characters) and ambiguously aligned regions (87 characters), 791 aligned characters were left: 161 were parsimony informative, 57 variable characters were parsimony uninformative and 543 were constant. The MP tree differed from the ML tree only in the placement of *Clavaria redoleo-alli* with *C. argillacea*, but this received weak support (–/–/61). A monophyletic group including the Clavariaceae s. str. (100/98/97) and *Mucronella* (99/94/93) received strong branch support (100/100/100). Two major lineages within the Clavariaceae s. str. also received strong support: /clavaria (100/93/75) and /ramariopsis (100/97/95). Each of these clades respectively represents the type species of *Clavaria* (*C. vermicularis*) and *Ramariopsis* (*R. kunzei*). However our analyses recover *Clavaria* and *Ramariopsis* (fide Petersen 1978) as polyphyletic. A classification of *Clavaria* (Petersen 1978) into subgen. *Syncoryne* (= subgen. *Clavaria*; *C. zollingeri*, *C. fumosa*, *C. vermicularis*-type) and sub-

gen. *Holocoryne* (*C. argillacea*, *C. redoleo-alli*) is rejected with moderate to strong support for paraphyly of the two subgenera (FIG. 5). *Clavaria* subgen. *Clavulinopsis* (*C. sulcata*-type) is recovered with *R. helvola* and *C. fusiformis* in /ramariopsis with moderate support (90/65/81).

Miscellaneous and incertae sedis. The genus *Lentaria* is paraphyletic with one isolate (*L. albovinacea*; GEL5388) in the euagarics grouped with *Cuphophyllus citrinopallidus* and *Chromosera cyanophylla* (/cuphophylloid clade 68; euMP, 11%), and the other isolate (*L. michneri*) from the Moncalvo et al (2002) dataset forming a monophyletic clade with the positively gravitropic clavarioid fungi *Kavinia* spp. within the gomphoid-phalloid clade (euMP, 30%). One isolate of *Kavinia* (*Kavinia* sp.; FO25092) is placed in a monophyletic group with the corticioid fungus *Coronicium alboglaucum* and the clavarioid fungus *Lachnocladium* sp. in the russuloid clade (euMP, 93%). Both isolates of *Clavulina cristata* (DJM1297 and one from Moncalvo et al 2002) and the corticioid fungus *Membrano-mycetes delectabilis* are resolved as a monophyletic group in the cantharelloid clade (euMP, 98%). A monophyletic group consistent with the athelioid clade is

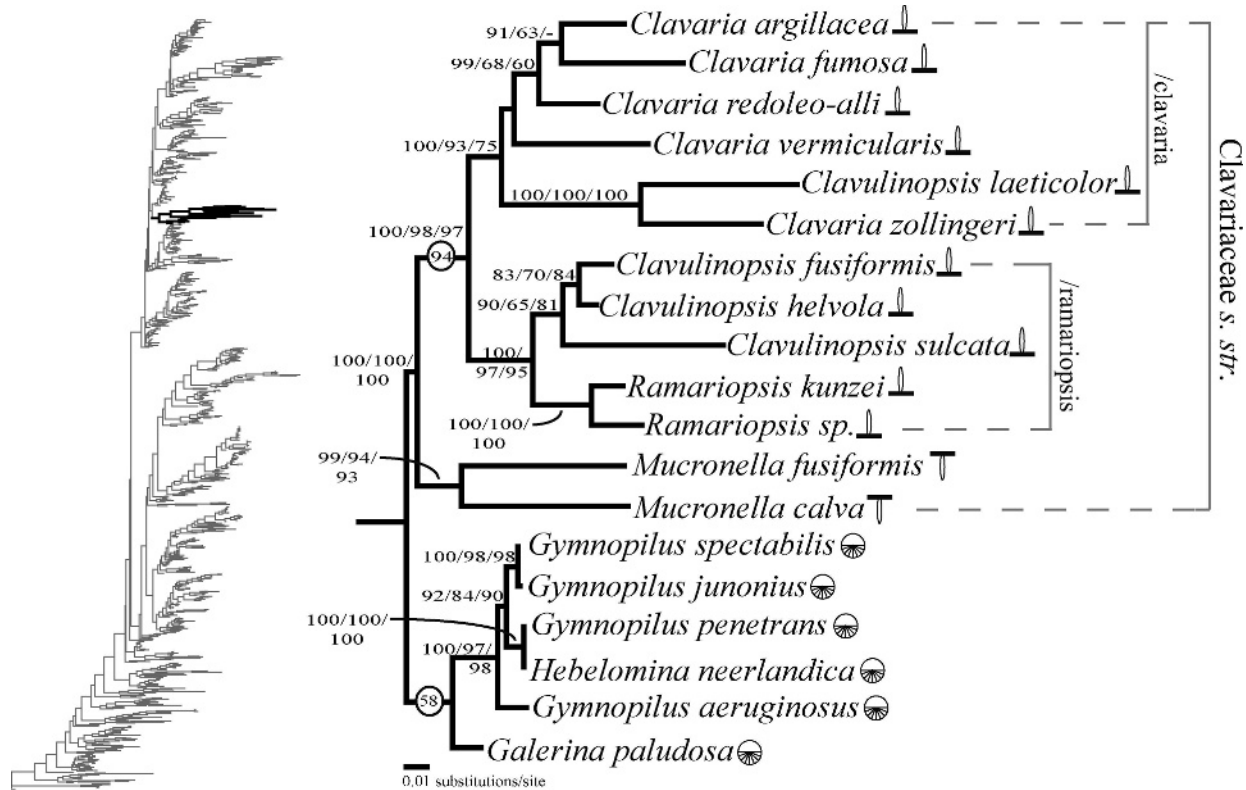


FIG. 5. A phylogram of one of 137 equally parsimonious trees of the Homobasidiomycetidae dataset is shown on the left with the branch leading to the “*Clavaria* clade” in bold. On the right is a maximum likelihood phylogram ($-\ln L$ 3707.2144) of the *Clavaria* clade after iterative TBR-swapping and re-estimation of model parameters using TrN+ Γ model. Rooted with outgroup *Gymnopilus spectabilis*, *G. junonius*, *G. penetrans*, *G. aeruginosus*, *Hebelomina neerlandica* and *Galerina paludosa*. Symbols after terminal taxa indicate fruit body morphology: clavarioid (\clubsuit), hydroid (\perp) and agaricoid (\oplus).

recovered containing *Athelia epiphylla*, *A. arachnoidea* (AF518601), *A. fibulata*, *Hyphoderma praetermissum* and *Pistillaria micans* (euMP, 15%). *Athelia arachnoidea* (AJ406484) is placed near *Podoscypha parvula* in the polyporoid clade (euMP, 18%).

DISCUSSION

Phylogenetic relationships.—*Hymenochaetoid clade.* Our results have unambiguously placed three geographically distant isolates of *Clavaria purpurea* in the /hymenochaetoid clade. To our knowledge this is the first report of the clavarioid morphology in the /hymenochaetoid clade using phylogenetic evidence. The affinity of *C. purpurea* to the hymenochaetoid fungi is surprising because the one characteristic it shares with the Hymenochaetaceae, clampless generative hyphae, is certainly not synapomorphic for the /hymenochaetoid clade. However many other members of the /hymenochaetoid clade previously classified in the Corticiaceae and Polyporaceae also lack the combination of characters used to distinguish the Hymenochaetaceae (Hibbett and Thorn 2000).

Of interest, *C. purpurea* and two allied species, *C. indica* and *C. nebulosoides*, are unique among *Clavaria* for possessing hymenial cystidia and the potentially related *Rickenella* is partially defined by possessing conspicuous pileo- and pleurocystidia (Singer 1986, treated in *Gerronema* subgen. *Romagnesia*). Comparative studies of cystidia at the light microscopic and ultrastructural levels of *Clavaria purpurea* and allies with *Rickenella* and *Omphalina* spp. might reveal whether phylogenetically informative characters exist in the structure or function of these cells.

Clavaria purpurea has been assumed to be saprotrophic, although no experimental studies support this view. The related hymenochaetoid *Coltricia perennis* is known to form ectomycorrhizae (Danielson 1984), while the potentially closely related *Rickenella*, *Cantharellopsis* and *Omphalina* are bryophilous. *C. purpurea* is found frequently fruiting among mosses (pers obs) and exclusively near coniferous trees (Corner 1950), a feature it shares with *C. nebulosoides* and which “should suffice to distinguish them among true *Clavarias*” (Corner 1950:248). These correlations suggest that the hypothesis that *C. purpurea* is saprotrophic should be

tested experimentally to rule out the possibility that it is mycorrhizal or bryophilous.

Although only 14 of an estimated 630 species of hymenochaetoid fungi (Hibbett and Thorn 2000) are included in this study, the sampling represents all known fruit body types in the clade. If our results can be confirmed with better sampled datasets then the phylogenetically proximal relationship to the diminutive agaric fungi *Rickenella*, *Cantharellopsis* and *Omphalina* in this study might indicate that *C. purpurea* represents a reduced fruit body type that was derived from agaricoid ancestors. However we caution against any firm conclusions drawn from this qualitative evaluation because branch support is weak and the sampling is incomplete.

We have shown that *C. purpurea* and the type species of *Clavaria* (*C. vermicularis*) do not share a recent common ancestor. Furthermore *Clavaria purpurea* is singular among related hymenochaetoid fungi for its possession of hymenial cystidia. The only other clavarioid fungi with potential affinities to the /hymenochaetoid clade are the lignicolous *Clavariachaete rubiginosum* and *C. peckoltii* from Amazonian Brazil and Venezuela, which possess setae and are allied to hydroid bracket fungi (Corner 1991). *Clavariachaete* and *Clavaria purpurea* share no characteristics except the clavarioid fruit body, which we have reaffirmed here is a highly homoplastic trait, and we have no reason to believe that they are closely related. Therefore the unique combination of characters for the /hymenochaetoid clade possessed by *C. purpurea* justifies recognizing it as a new, independent genus.

Alloclavaria B. Dentinger et D. J. McLaughlin, gen. nov.

Etymology. Meaning “the other *Clavaria*,” referring to the morphologically similar genus and basionym of the type species.

Alloclavaria continet fungos, vel clavarioides vel coraloides, qui possident hymenialia cystidia parietibus tenuibus et orti sunt monophyletico clado ab Hymenochaetalibus. Typus generis: *Alloclavaria purpurea* (Fr.) B. Dentinger & D.J. McLaughlin.

Alloclavaria includes club-like and coral-like mushrooms that have thin-walled hymenial cystidia and form a monophyletic clade in the Hymenochaetales.

Type of genus. *Alloclavaria purpurea* (Fr.) B. Dentinger & D.J. McLaughlin.

This diagnosis is both phylogenetic and morphological. We expect that additional species may be allied to *Alloclavaria purpurea* (e.g., *Clavaria indica* and *C. nebulosoides*).

Alloclavaria purpurea (Fr.) B. Dentinger et D.J. McLaughlin, comb. nov.

Basionym: *Clavaria purpurea* Fr. *Syst. mycol.* (Lundae) 1:480. 1821.

Macrotyphula clade. Our analyses have resolved with strong support (euMP, 85%) a monophyletic group containing the clavarioid fungi *Macrotyphula* and *Typhula*, including the type species of each, *M. fistulosa* and *T. phacorrhiza*. In the only monograph dedicated to these clavarioid genera, *Macrotyphula* and *Typhula* form a natural group with *Ceratellopsis* based on morphological features (Berthier 1976).

Macrotyphula was erected by Petersen to accommodate *Clavariadelphus fistulosus* (Fr.) Corner (*Clavariadelphus* subgenus *Typhulopsis*), which he considered “to be a discordant element in the genus” (Petersen 1972:138). Berthier recognized *Macrotyphula* as separate from the other two typhuloid genera based on its large size and lack of a sclerotium (Berthier 1976). In contrast Berthier maintained a broad definition of *Typhula*, lumping these together as subgenera: *Typhula* Fr., *Pistillina* Quél., *Gliocoryne* Maire, *Pistillaria* Fr., *Typhulina* Berthier et Khurana, *Microtyphula* Berthier, and *Cnazonaria* Corda (Berthier 1976). Although Corner treated *P. micans* as the type of *Pistillaria*, Berthier’s concept was centered on *Typhula*. Consequently Berthier recognized *T. quisquiliaris* (Fr.) Henn. as the type of subgen. *Pistillaria*, and although he transferred *P. micans* to *Typhula* he recognized its distinctness by allying it with a small assemblage of incongruent species “ne s’insérant pas dans les sous-genres distingués par l’auteur” (Berthier 1976:163). We have included one isolate of the type species of *Pistillaria* (*P. micans*) but were able only to recover a weakly supported affinity to athelioid fungi (euMP, 15%). Although unresolved *P. micans* might represent yet another independent origin of the clavarioid morphology in the Homobasidiomycetidae.

The /macrotyphula clade appears to share a recent common ancestor with the /phyllotopsis clade (euMP, 61%) containing the agaric fungi *Phyllotopsis nidulans* and *Pleurocybella porrigens* and the cypheloid fungus *Henningsomyces candidus*. These results are consistent with Moncalvo et al (2002) and Binder et al (2005). Larsson et al (2004) recovered a relationship between *Macrotyphula* and *Typhula* but failed to include any representatives of /phyllotopsis and instead recovered an unsupported relationship to corticioid fungi. Our results did not recover this relationship even though we included all of the taxa resolved in the sister group by Larsson et al (2004). Our analyses included two geographically distant isolates of *Macrotyphula juncea* with identical nucLSU rDNA sequences, one from Puerto Rico (DJM1032) and one from Costa Rica (DJM975), and sequences of three isolates of *Typhula phacorrhiza* from GenBank.

We also found a strongly supported relationship between *Macrotyphula fistulosa* and “*Bulbillomyces farinosus*.” If this is true then *Macrotyphula* is paraphyletic. However the authenticity of the *Bulbillomyces* sequence is dubious (Binder et al 2005), and better documented isolates are needed before a firm conclusion can be made. Multiple fruit body types have evolved in this group, but due to incomplete sampling the evolutionary significance of the clavarioid morphology cannot be determined adequately.

Chaetotyphula clade. This strongly supported clade of small clavarioid fungi appears to be related to agaricoid (*Hemimycena* spp.) and cyphelloid (*Calcyptella capula*) fungi. The /chaetotyphula clade is composed of representatives of at least two genera in two traditional families: Clavariaceae (*Chaetotyphula*) and Pterulaceae (*Actiniceps*). We have included the type species of *Chaetotyphula* (*C. hyalina*) and two isolates that were not assignable to known species (BDCR0419, DJM1031). These small typhuloid fungi are allied by their conspicuous cystidia but separated by the absence (*Chaetotyphula*) or presence (*Actiniceps*) of skeletal hyphae. While the Pterulaceae is defined partly by the presence of skeletal hyphae, our results indicate that this character is not a reliable synapomorphy for the family. At the present time the only morphological characters that unite *Chaetotyphula* and *Actiniceps* are their small size, absence of pigments, presence of hymenial- and/or caulocystidia, and hyaline, smooth-walled, globose to ellipsoid spores. The limits of this group are not well established, and these inconspicuous fungi might be more diverse than we currently appreciate.

Although support was weak (69/59/-), the intermediate position of the cyphelloid *Calcyptella capula* between the agaricoid fungi *Hemimycena* spp. and /chaetotyphula suggests an intriguing evolutionary hypothesis: The cyphelloid morphology might represent an intermediate stage in the reduction of agaricoid fungi to clavarioid fungi. However more data and critical evaluations of alternate evolutionary scenarios are essential to draw any firm conclusions regarding the evolution of the clavarioid fruit body in this series of fungi.

Physalacriaceae clade. *Physalacria* was first erected by C.H. Peck to “correct” the placement of the temperate species *Mitrella inflata* Schwein.:Fr. (Peck 1882). Peck placed *P. inflata* in the Clavariaceae by a phenetic correlation of the “simple” fruit body types, taking as evidence the nearly uniform distribution of the hymenium over the surface of the fruit body (or pileus, in the case of *Physalacria*). However the observation of irregularities in the distribution of the hymenium has led to various interpretations of the classification of the physalacrioid species. Krieger

(1923) considered the simple fruit body morphology and the folds on the underside of the pileus of *P. inflata* to represent the characters of the ancestral form of agaric fungi. He accommodated his vision by changing the generic name to *Eoagaricus* and creating the family Eoagaricaceae. Using cytological evidence to reject Krieger’s speculations, *Physalacria* later was transferred into the Thelephoraceae (McGuire 1939) and then returned to the Clavariaceae (Baker 1941). More recently Singer (1962) and Donk (1964) proposed a new concept of the physalacrioid fungi by placing them in the Agaricales near *Marasmius*. Although Corner (1970) recognized the microanatomical correlation of the physalacrioid species with the marasmioid fungi, he rejected this classification and instead created the Physalacriaceae, *incertae sedis*. In the most recent monograph of physalacrioid fungi Berthier (1985) accepted Corner’s classification and included three genera, *Physalacria*, *Hormomitaria* and *Pseudotyphula*. In the last edition of *The Agaricales in Modern Taxonomy* (1986), Singer preserved his classification of the physalacrioid fungi in the subtribe Marasmiinae (tribus Marasmieae, Tricholomataceae, Agaricales).

The unusual physalacrioid fruit body might have evolved through convergence. Seven isolates of *Physalacria* spp. are placed in three different positions in the Homobasidiomycetidae. At least one of these isolates, “*P. inflata*” (HHB-13443-sp), was placed in the polyporoid clade near either *Wolfiporia cocos* (Binder et al 2005) or *Irpex lacteus* and *Trametes suaveolens* (this study). We suspect that the sequence from this cultured isolate is identified incorrectly because the sequence of *P. inflata* (BD347) was generated from a recently collected and positively identified fruit body. This is the first time that the type species of *Physalacria* has been confidently placed in this clade.

The second independently placed *Physalacria* sequence is from an unidentified isolate (GEL5189). In our analysis this isolate is closely related to *Marasmius capillaris* and *M. rotula* of the /marasmioid clade, which is near the /physalacria clade in Moncalvo et al (2002) and this study. *Marasmius capillaris* and *M. rotula* are classified in sect. *Marasmius*, subsect. *Pararotulae*, which is defined by those species with an epicutis containing broom cells with divergent setulae (Singer 1986). It is notable that some species of *Physalacria* have distinct broom cells like *M. capillaris* and *M. rotula*, while others do not. In this study all species sampled from the /physalacria clade lack these characteristic broom cells, while it is not known whether the singular isolate, GEL5189 (if it is a *Physalacria* sp.), has this type of cell. Nonetheless this distinct morphological feature presents an

interesting division in *Physalacria* that is worth investigating more thoroughly.

Finally, our secondary analyses strongly support *Physalacria inflata* with *Gloiocephala spathularia* (100/100/100) in a sister position to the other *Physalacria* isolates. This intriguing result suggests that the flabellate-spathulate morphology of *Gloiocephala spathularia* might represent an evolutionarily intermediate form with an uninflated enlargement of the apex (or rudimentary pileus) apparent in the transition from either agaricoid or physalacrioid ancestors. Thus the physalacrioid morphology might have arisen through parallel evolution or reversal to the ancestral condition, two hypotheses worth revisiting once more data are available.

We have demonstrated that this well supported clade does contain the type species of *Physalacria* (*P. inflata*). Consequently we propose to follow the tentative classification of the Physalacriaceae by Moncalvo et al (2002) to include these species: *Armillaria irazuensis*, *Armillaria* "NABS I," *Armillaria tabescens*, *Cyptotrama asprata*, *Flammulina velutipes*, *Gloiocephala menieri*, *Gloiocephala* sp. (TENN7573), *Gloiocephala spathularia*, *Oudemansiella canarii*, *Physalacria inflata*, *Physalacria maipoensis*, *Physalacria* aff. *orinocensis*, *Physalacria orinocensis* (DJM1035), *Rhizomarasmius pyrrocephalus*, *Rhodotus palmatus*, *Strobilurus trullisatus*, *Xerula furfuracea* and *Xerula megalospora*. More work is needed to clarify the limits of and relationships within the Physalacriaceae, but several previously recognized features might be synapomorphic. For instance most of these taxa have a pileipellis composed of clavate cells embedded in a gelatin-like matrix and are lignicolous (Moncalvo et al 2002).

Clavaria clade. The euagaric dataset resolved *Phlebia tristis* with *Mucronella*. Therefore we initially included *P. tristis* in our secondary matrix. When analyzed with MP, ML and BA methods *P. tristis* always was placed with *Mucronella* on a long branch. Upon removal of *P. tristis* branch support leading to *Mucronella* and Clavariaceae *s. str.* increased. These results indicated that the placement of *Phlebia tristis* with *Mucronella* was misleading and likely a result of a misidentified specimen or culture, a chimeric sequence or "long-branch attraction" (Felsenstein 1978). We have presented only the results of analyses with *P. tristis* excluded.

For the first time our analyses have recovered a close relationship between the positively gravitropic clavarioid genus *Mucronella* and the negatively gravitropic family Clavariaceae and have identified the /gymnopiloid clade as the sister lineage to the Clavariaceae and *Mucronella* clades. The close relationship of positively (Clavariaceae) and negatively (*Mucronella*)

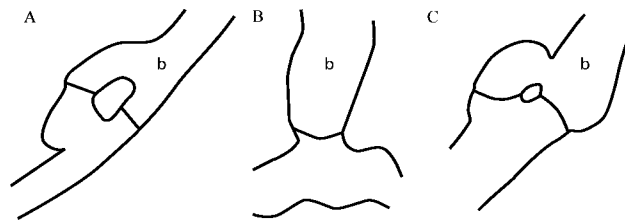


FIG. 6. Clamp connections used to define three subgenera of *Clavaria* (fide Petersen 1978). Line traces from digital photos (2000 \times) of the base of the basidium (b) taken from dried fruit bodies rehydrated in 5% KOH. A) *Clavaria* subgen. *Holocoryne* (*C. redoleo-alli*, DJM1079), B) *Clavaria* subgen. *Syncoryne* (*C. vermicularis*, DJM1262), C) *Clavaria* subgen. *Clavulinopsis* (*C. sulcata*, PDD78241).

gravitropic lineages is intriguing and parallels the affinities of *Pterula* and *Deflexula* in the Pterulaceae (Munkacsy et al 2004). The lignicolous habit of *Mucronella* might indicate also that the ancestor of the Clavariaceae was a wood-dwelling saprotroph.

Corner's treatment of *Clavaria s. str.* included two "natural" groups that he classified into two subgenera: *Clavaria* (= *Syncoryne*) and *Holocoryne*. This arrangement was based on morphology of the clamp connections, where subgen. *Holocoryne* was delimited by clampless hyphae and a bifurcating ("wide loop-like") clamp at the base of the basidium, while subgen. *Clavaria* lacked clamps entirely (FIG. 6). Corner defined *Ramariopsis* by branched fruitbodies with clamps and echinulate spores, and *Clavulinopsis* by two forms: (i) branched fruit bodies with clamps and smooth spores or (ii) simple fruit bodies with clamps and echinulate spores (Corner 1950, 1970). In a reorganization of the four genera Petersen split members of the genus *Clavulinopsis* between *Clavaria* and *Ramariopsis* Petersen (1978). Although he recognized the developmental plasticity of the clamp morphology Petersen (1978) continued to adopt Corner's emphasis on this character in an alternative classification. According to Petersen (1978) three subgenera of *Clavaria* could be recognized: subgen. *Clavaria*, subgen. *Holocoryne* (fide Corner 1950, 1970) and subgen. *Clavulinopsis*, the latter of which included taxa with non-*Holocoryne*-type clamps on the hyphae and at the base of the basidium (FIG. 6). Petersen considered subgen. *Clavulinopsis* to be allied to subgen. *Clavaria* based on the shared absence of the *Holocoryne*-type clamp. Corner rejected this rearrangement and maintained *Clavulinopsis* as a genus separate from both *Clavaria* and *Ramariopsis*, although he admitted the proximity of *Clavulinopsis* to *Ramariopsis* and speculated on a broader classification given further evidence (Corner 1970). On the other hand Corner recognized three subgenera in *Clavulinopsis*: *Acularia* with

Taxon	Corner (1950, 1970)	Petersen (1978)	Spore shape	Spore texture	Fruitbody elaboration	Fruitbody color
<i>Clavaria argillacea</i>	H	H	ellipsoid- subcylindric	smooth	simple	white
<i>Clavaria fumosa</i>	Ca	Ca	ellipsoid- pip- shaped	smooth	simple	white
<i>Clavaria redoleo-alli</i>	H	H	subglobose- broadly o.vate	smooth	simple	white
<i>Clavaria vermicularis</i>	Ca	Ca	ellipsoid or pip-shaped	smooth	simple	white
<i>Clavulinopsis/Ramariopsis laeticolor</i>	Cs (subgen. Clavulinopsis sect. Cornicularia)	R (subgen. Laevispora)	elongate-ovate to elongate triangular	smooth	simple	yellow/orange
<i>Clavaria zollingeri</i>	Ca	Ca	subglobose- broadly ellipsoid; pip- shaped	smooth	branched	purple
<i>Clavulinopsis/Ramariopsis fusiformis</i>	Cs (subgen. Clavulinopsis sect. Cornicularia)	R (subgen. Laevispora)	subglobose- broadly ellipsoid	smooth	simple	yellow
<i>Clavulinopsis/Ramariopsis helvola</i>	Cs (subgen. Acularia)	R (subgen. Ramariopsis)	subglobose	echinulate	simple	yellow/orange
<i>Clavulinopsis/Clavaria sulcata</i>	Cs (subgen. Clavulinopsis sect. Clavulinopsis)	Cs	globose- subglobose	smooth	simple	yellow/orange
<i>Ramariopsis kunzei</i>	R	R (subgen. Ramariopsis)	globose- subglobose	echinulate	branched	white
<i>Ramariopsis sp.</i> (DJM1047)	R	R (subgen. Ramariopsis)	globose- subglobose	echinulate	branched	white

FIG. 7. A comparison of competing classifications of *Clavaria*, *Clavulinopsis* and *Ramariopsis* with selected morphological characters. Cladogram on the left side is a summary of relationships that received strong support from our analyses (FIG. 5). Abbreviated taxonomic names are subgen. *Clavaria* (Ca), subgenus *Holocoryne* (H), genus (Corner) or subgen. (Petersen) *Clavulinopsis* (Cs), genus *Ramariopsis* (R). Morphological characters are taken from (Corner 1950, 1970) and (Petersen 1988).

ornamented spores and *Clavulinopsis* and *Paraclavaria* with smooth spores. The latter two were differentiated by shape (subglobose to ovoid in subgen. *Clavulinopsis* vs. ellipsoid in subgen. *Paraclavaria*) or by the presence of a prominent apiculus (1–2 μm long) in those members of subgen. *Clavulinopsis* with ellipsoid spores.

Our results are partially consistent with the classification of Corner (1970) and partially consistent with that of Petersen (1978). However our sampling is too limited to draw many firm conclusions. Nonetheless, with the exception of *Ramariopsis laeticolor*, our results do support monophyly of *Clavaria* and recognition of *Clavulinopsis* at the generic level as proposed by Corner and reinforce the affinity of *Clavulinopsis* with *Ramariopsis* (FIGS. 5 and 7). With our sampling the distribution of morphological characters that are most consistent with the well supported nodes include fruit body color and branched vs. simple fruit bodies in

/ramariopsis, while the distribution of clamp connection morphology, spore shape and spore wall texture is not clearly correlated with phylogenetic relationships (FIG. 7).

We propose a revision of the family Clavariaceae to reflect a single ancestor of the type species *Clavaria vermicularis* and all of its descendents. Although we have not included *Scytinopogon* in our survey we predict it will be allied with the telephoroid fungi. We tentatively exclude this genus from the Clavariaceae but point out that Corner treated it with ambiguous affinities to telephoroid fungi and *Ramariopsis* (Corner 1970). The phylogenetic placement of this genus needs to be addressed.

The most appropriate ancestor to represent the origin of the Clavariaceae is at the strongly supported node leading to all isolates of clavarioid fungi of the ingroup containing the type species of *Clavaria*. In our analysis this also includes the type species of

Clavulinopsis, *Mucronella*, and *Ramariopsis* (FIG. 5). To our knowledge this is the first time that the Clavariaceae *sensu stricto* is recovered in a phylogenetic analysis. At this time it is difficult to determine the generic limits of *Clavaria*, *Clavulinopsis* and *Ramariopsis*. Our results do not entirely support the classifications of Corner (1950, 1970) or Petersen (1978) but indicate strong support for a sister relationship of *Clavaria* with *Ramariopsis*.

CONCLUSIONS

Homobasidiomycete systematics has undergone an important revolution with the application of molecular techniques in the early 1990s. Classifications originally based on morphological and biochemical characters are being transformed, reflecting contemporary ideas and evidence from DNA sequences. However authenticity of DNA sequences remains a problem. While the value of living cultures as an extension of the traditional herbarium cannot be overemphasized, it is evident that sequences derived from vegetative cultures that give unexpected results be verified, when possible, with sequences obtained from identifiable fruit bodies. In this study such an approach is analogous to including multiple and geographically distant isolates of *Clavaria purpurea* to improve our confidence in its unexpected phylogenetic position.

However large and publicly available datasets with widely sampled, reliably identified and properly vouchered specimens, such as that of Moncalvo et al (2002), WASABI (www.aftol.duke.edu/wasabi) and UNITE (Koljalg et al 2005), are indispensable tools in systematic mycology. These datasets let researchers evaluate the phylogenetic distributions of many different taxa simultaneously and can illuminate relationships that remain cryptic with traditional methods. In this paper we have demonstrated how an analysis of clavarioid fungi within the broad context of the Homobasidiomycetidae was essential to identify the phylogenetic position of five unrelated clades of coral mushrooms and reveal the unexpected placement of *Clavaria purpurea* within the hymenochaetoid clade. Without such broadly sampled datasets our phylogenetic reconstructions might have indicated strong conflict between the positions of *C. purpurea* and its supposed morphological relatives but would not have enabled us to identify its affinities to the hymenochaetoid fungi.

This research reveals widespread convergence on the clavarioid morphology. Moreover it points to an apparent tendency for the derivation of the clavarioid morphology from agaricoid or corticioid ancestors. However the extremely limited sampling of homo-

basidiomycete taxa that is currently available, and especially of the clavarioid forms, means that interpreting these trends might be premature. Only once additional data are available, and the fungal tree of life becomes more predictable, will revisiting these ideas be tractable.

The correlation between the abundance of clavarioid taxa in high moisture habitats, particularly in the tropics, and the polyphyletic history of these taxa is evidence for adaptive convergence on the clavarioid morphology. How might the clavarioid morphology be adaptive? Perhaps there is an advantage to the short development times of coral mushrooms, a resistance to hyperhydration and premature decay by limiting the amount of tissue used in construction of the fruit body or economical use of limited resources in a competitive environment. In addition, even though an analysis of trends in fruitbody evolution seems premature, our results contradict earlier interpretations that the clavarioid morphology is evolutionarily labile (Hibbett 2004) but that there might be a trend for clavarioid forms to be derived. These questions can be addressed only with a more complete knowledge of the global Eumycota, and to this end we underscore the need for more documentation and phylogenetic incorporation of the poorly known neo- and paleotropical fungi.

Clavaria vermicularis Fr. is a synonym of *C. fragilis* Holmsk. (Index Fungorum, www.indexfungorum.org, 6 Nov 2006).

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