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Phylogenetic relationships of cyphelloid homobasidiomycetes

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Abstract

The homobasidiomycetes includes the mushroom-forming fungi. Members of the homobasidiomycetes produce the largest, most complex fruiting bodies in the fungi, such as gilled mushrooms (''agarics''), boletes, polypores, and puffballs. The homobasidiomycetes also includes species that produce minute, cup- or tube-shaped ''cyphelloid'' fruiting bodies, that rarely exceed 1–2 mm diameter. The goal of this study was to estimate the phylogenetic placements of cyphelloid fungi within the homobasidiomycetes. Sequences from the nuclear large subunit (nuc-lsu) ribosomal DNA (rDNA), 5.8S rDNA, and internal transcribed spacers (ITS) 1 and 2 were obtained for 31 samples of cyphelloid fungi and 16 samples of other homobasidiomycetes, and combined with published sequences. In total, 71 sequences of cyphelloid fungi were included, representing 16 genera. Preliminary phylogenetic analyses of a 1477-sequence data set and BLAST searches using sequences of cyphelloid forms as queries were used to identify taxa that could be close relatives of cyphelloid forms. Subsequent phylogenetic analyses of one data set with 209 samples represented by nuc-lsu rDNA sequences (analyzed with parsimony) and another with 38 samples represented by nuc-lsu and 5.8S rDNA sequences (analyzed with parsimony and maximum likelihood) indicated that cyphelloid forms represent a polyphyletic assemblage of reduced agarics (euagarics clade, Agaricales). Unconstrained tree topologies suggest that there have been about 10–12 origins of cyphelloid forms, but evaluation of constrained topologies with the Shimodaira–Hasegawa test suggests that somewhat more parsimonious scenarios cannot be rejected. Whatever their number, the multiple independent origins of cyphelloid forms represent striking cases of parallel evolutionary reduction of complex fungal morphology.

Keywords: Homobasidiomycetes; Agarics; Euagarics clade; Cyphelloid fungi; Evolutionary reduction

1. Introduction

Evolutionary reduction is the derivation of relatively small, morphologically and anatomically simple organisms from larger, more complex ancestors. Reduction poses challenges for both taxonomists and evolutionary theorists. For taxonomists, reduced organisms are difficult because they lack many of the characters that are present in their unreduced relatives. For evolutionary theorists, understanding the prevalence of reduction is central to determining whether there are general evolutionary trends toward increasing size or complexity of organisms. Most discussions about reduction have concerned animals (e.g., Jablonski, 1997; Sidor, 2001). The present study, describes examples of evolutionary reduction in the homobasidiomycetes (mushroom-forming fungi).

The homobasidiomycetes is the most conspicuous group of fungi, including approximately 16,000

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described species of mushroom-forming fungi and related forms (Kirk et al., 2001; http://tolweb.org/tree?group= Homobasidiomycetes&contgroup=Hymenomycetes). Familiar examples of homobasidiomycetes include gilled mushrooms, polypores, and puffballs. Besides these, homobasidiomycetes also contain a relatively obscure assemblage of so-called "cyphelloid" fungi. Cyphelloid fungi have minute, cup- to barrel-shaped or tubular, often pendant fruiting bodies that are typically less than 2 mm in length and diameter (rarely exceeding 1 cm). Cyphelloid fruiting bodies usually have a smooth, even hymenophore (spore-producing-surface) that lines their concave inner surface (Agerer, 1978a, 1983a; Donk, 1951, 1959, 1966). Morphology and anatomy provide relatively few taxonomically informative characters for the classification of cyphelloid forms. Traditionally, these characters were mostly derived from spore morphology and anatomy of hyphae that cover the external surface of the fruiting bodies (Agerer, 1973, 1975, 1983b, 1986b; Cooke, 1962; Donk, 1966). Cyphelloid fungi include roughly 120 anatomically well-characterized taxa that have been accommodated in ca. 40 widely accepted genera (Agerer, 1983b; Cooke, 1962; Donk, 1959; Reid, 1964; Singer, 1986). Their actual diversity is still unknown, but Agerer (personal communication) estimates that there could be as many as 400–500 cyphelloid species.

Cyphelloid forms have been grouped in the artificial family "Cyphellaceae," and this term is still in use as a matter of convenience. However, it is generally accepted that cyphelloid fungi are polyphyletic (Agerer, 1986b; Donk, 1951, 1959, 1962, 1971; Singer, 1986). Relationships have been suggested with diverse forms of homobasidiomycetes, including pileate agarics with lamellate (gilled) hymenophores, corticioid (crust-like) fungi with smooth hymenophores, and polypores with tubular hymenophores (Agerer, 1978a; Bondarzew and Singer, 1941; Cooke, 1962, 1989; Donk, 1959; Horak and Desjardin, 1994; Singer, 1966, 1986). The majority of cyphelloid genera have anatomical similarities to various genera of agarics. It has been supposed by different authors (Agerer, 1978a; Donk, 1959; Horak and Desjardin, 1994; Singer, 1966, 1986) that cyphelloid fruiting bodies have evolved multiple times by reduction from agaricoid ancestors.

Only a few phylogenetic studies have included any cyphelloid homobasidiomycetes (Binder et al., 2001; Hibbett and Binder, 2001; Langer, 2002; Moncalvo et al., 2002), and none have focused specifically on cyphelloid forms. Binder et al. (2001), Hibbett and Binder (2001) demonstrated that certain cyphelloid fungi are related to marine homobasidiomycetes, but the relationships of most cyphelloid fungi have not been determined. The present study is the first to focus primarily on the phylogenetic relationships of cyphelloid fungi.

2. Materials and methods

2.1. Taxon sampling and target genes

The taxa sampled in this study included cyphelloid forms, taxa that have been suggested to be related to cyphelloid fungi based on their anatomical characters (Agerer, 1978a, 1983b; Donk, 1962, 1971; Singer, 1986), and representatives of other groups of homobasidiomycetes. When possible, multiple samples of individual species were analyzed to verify the generated sequences and their placement. The genes that were targeted include partial nuc-lsu rDNA, bounded by primers LR0R (ACCCGCTGAACTTAAGC) and LR5 (TCCTGAGGGAAACTTCG), and the ITS rDNA region, bounded by primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) (Gardes and Bruns, 1993; Vilgalys and Hester, 1990; White et al., 1990; for primer sequences see http://plantbio.berkeley.edu/~bruns/primers.html and http://www. biology.duke.edu/fungi/mycolab/primers.htm).

DNA isolation and PCR amplification was attempted using 78 cyphelloid and 20 non-cyphelloid samples. Ultimately, 78 sequences were generated from 47 samples, representing the cyphelloid genera *Amyloflagellula*, *Calathella*, *Calyptella*, *Cyphellopsis*, *Flagelloscypha*, *Halocyphina*, *Henningsomyces*, *Lachnella*, *Merismodes*, *Pellidiscus*, *Phaeosolenia*, *Rectipilus*, *Stigmatolemma*, and *Woldmaria*, and 16 other homobasidiomycetes (Table 1). Fourteen previously published sequences of cyphelloid fungi were downloaded from GenBank, including sequences of the genera *Cyphella* and *Stromatoscypha*, for which no new sequences were generated.

To select additional taxa, a series of preliminary phylogenetic analyses and BLAST searches were conducted. The goal of these preliminary analyses was to identify species that could be closely related to cyphelloid forms. The preliminary phylogenetic analyses used a 1477-sequence reference data set containing unpublished and published sequences that represent all major groups of homobasidiomycetes, with an emphasis on the euagarics clade (Hibbett and Thorn, 2001; Moncalvo et al., 2002). The reference data set included overlapping data for four rDNA regions (nuclear and mitochondrial small and large subunit rDNA), but every species in the reference data set was represented by nuc-lsu rDNA. The reference data set contained roughly 60% of the approximately 2500 nuc-lsu rDNA sequences currently present in GenBank. Preliminary analyses were performed using maximum parsimony (MP) and bootstrapped neighbor-joining (NJ) (results are not shown, but are available on request). BLAST searches were performed using nuc-lsu and ITS rDNA sequences of representatives of all sampled cyphelloid genera as queries.

Based on the results of the preliminary analyses, a set of 168 nuc-lsu rDNA sequences was selected for combi-

Table 1
New generated sequences in this study: taxon, isolate code, country of origin, and GenBank accession numbers

Taxon	Isolate code[a]	Country of origin	GenBank Accession Nos.	
			nuc-lsu rDNA	ITS
Cyphelloid forms				
Amyloflagellula inflata	PB305/RA	Reunion (France)	AY570990	AY571027
Calathella columbiana	PB327/RA	Colombia	AY570993	AY571028
Calathella gayana	ZT8836	Chile	AY572005	AY572009
Calathella mangrovei	1-31-01 Jones	Malaysia		AY571029
Calyptella capula	CBS485.86	Netherlands	AY570994	AY571030
Calyptella capula	PB315	Norway	AY570995	AY571031
Cyphellopsis anomala	PB318	Germany	AY570998	AY571035
Cyphellopsis anomala	PB323	Germany	AY570999	AY571036
Cyphellopsis anomala	PB333	Germany	AY571000	AY571037
Cyphellopsis anomala	CBS151.79	Netherlands		AY571034
Flagelloscypha minutissima	CBS823.88	Germany	AY571006	AY571040
Flagelloscypha sp.	Horak9544	USA	AY571007	AY571041
Halocyphina villosa	IFO32088	Japan		AY571042
Henningsomyces sp.	C58569	Ecuador	AY571011	AY571046
Henningsomyces sp.	FP-105017-Sp	USA	AY571010	AY571047
Henningsomyces puber	GUA-307	Guana	AY571009	AY571045
Henningsomyces candidus	PB338	France	AY571008	AY571044
Henningsomyces candidus	T156	Canada		AY571043
Lachnella alboviolascens	PB332	Germany	AY571012	AY571048
Lachnella villosa	PB321	Germany	AY571013	AY571049
Lachnella villosa	PB322	Germany	AY571014	AY571050
Merismodes fasciculata	HHB-11894	USA	AY571015	AY571051
Merismodes fasciculata	PB342	Switzerland	AY571016	AY571052
Pellidiscus pallidus	C58178	Ecuador	AY571017	AY571054
Phaeosolenia densa	C61839	Ecuador	AY571018	AY571055
Phaeosolenia densa	C61963	Ecuador	AY571019	AY571056
Rectipilus idahoensis	PB313/RA	Reunion (France)	AY571020	AY571057
Rectipilus natalensis	PB312/RA	Reunion (France)	AY571021	AY571058
Stigmatolemma conspersum	C61852	Ecuador	AY571024	AY571061
Stigmatolemma poriaeforme	CBS327.91	Canada	AY571025	AY571062
Woldmaria crocea	NH-10.23.95	Sweden	AY571026	
Other forms				
Auriculariopsis ampla	NH-1478 Romania	Romania	AY570991	
Auriculariopsis ampla	CBS228.97	Netherlands	AY570992	
Chaetocalathus liliputianus	C61867	Ecuador	AY570996	AY571032
Crinipellis stipitaria	PB302	Germany	AY570997	AY571033
"Entoloma lividum?"	MB5034	USA	AY571001	
Favolaschia calocera	PDD70689	New Zealand	AY572006	
Favolaschia calocera	PDD71528	New Zealand	AY572007	
Favolaschia pezizaeformis	PDD67440	New Zealand	AY572008	
Fistulina antarctica	REG550	Chile	AY571002	
Fistulina endoxantha	CIEFAP115	Argentina	AY571003	
Fistulina hepatica	REG593	USA	AY571004	AY571038
Fistulina pallida	CBS508.63	USA	AY571005	AY571039
Nia vibrissa	REG M200	Turkey		AY571053
Porodisculus pendulus	HHB-13576-Sp	USA		AY572009
Resupinatus applicatus	PB335	France	AY571022	AY571059
Schizophyllum radiatum	CBS301.32	Panama	AY571023	AY571060

[a] *Abbreviations:* C, Herbarium University of Copenhagen, Copenhagen, Denmark; CBS, Centraalbureau voor Schimmelcultures, Baarn, Netherlands; CIEFAP, Andean-patagonian Forestry Research and Advisory Center, Chubut, Argentina; FP, Forest Products Laboratory, Madison, Wisconsin, USA; HHB, personal herbarium of H. H. Burdsall; Horak, personal herbarium of E. Horak; IFO, Institute for Fermentation, Osaka, Japan; NH, personal herbarium of N. Hallenberg, Goeteborg, Sweden; PB, personal herbarium of P. Bodensteiner, Munich, Germany; PDD, New Zealand Fungal Herbarium, Landcare Research, Auckland, New Zealand; RA, personal herbarium of R. Agerer, Munich, Germany; REG, Herbarium, Regensburgische Botanische Gesellschaft Universität Regensburg, Regensburg, Germany; T, personal herbarium of G. Thorn; ZT, Herbarium Eidgenössische Technische Hochschule Zürich, Zürich, Switzerland.

nation with the new sequences. These sequences represent putative close relatives of cyphelloid fungi and other groups in the euagarics clade, as well as members of the bolete clade (*Boletus satanas*, *Coniophora olivacea*, *Paxillus involutus*, and *Suillus cavipes*), and *Jaapia argillacea*, which was used for rooting purposes. Inclusion of

the boletes and *J. argillacea* was based on the results of previous analyses, which suggested that the bolete clade is the sister group of the euagarics clade and *J. argillacea* is the sister group of the bolete clade plus euagarics clade (Binder and Hibbett, 2002; Hibbett and Binder, 2002).

2.2. Molecular techniques, alignment, and phylogenetic analyses

DNA was extracted from cyphelloid herbarium specimens using the E.Z.N.A. Forensic DNA Kit (Omega Bio-tek), which is optimized for minute specimens, following the manufacturer's instructions. DNA from cultures was obtained by using a phenol–chloroform extraction procedure (Lee and Taylor, 1990). Protocols for polymerase chain reaction (PCR) amplification and sequencing have been described elsewhere (Vilgalys and Hester, 1990; White et al., 1990). The nuc-lsu and ITS rDNA regions were amplified and sequenced directly using dye-terminator cycle-sequencing chemistry (Applied Biosystems). Sequencing reactions were run on an ABI377XL automated DNA sequencer. Sequences were edited and contiguous sequences were assembled using ABI analysis software and Sequencher version 4.1 (Gene Codes Corporation). DNA sequences were aligned by eye in the data editor of PAUP* 4.0b10 (Swofford, 2002).

Two sets of phylogenetic analyses were performed using two different data sets: Data set I included nuc-lsu rDNA sequences of 209 samples, representing diverse groups within the euagarics clade. Data set II included nuc-lsu rDNA and 5.8S rDNA sequences from a subset of 38 taxa from data set I, representing cyphelloid fungi and closely related agarics. Both alignments have been deposited in TreeBASE (SN1112,M1902-M1903).

Analyses of data set I used maximum parsimony with all characters included and treated as unordered and equally weighted. The analyses were performed according to a two-step search protocol that has been described by Hibbett and Donoghue (1995). Step one involved 1000 heuristic searches with random taxon addition sequences and TBR branch swapping while keeping only two trees per replicate. The shortest trees retained in the first step were used as starting trees in step two with TBR branch swapping and MAXTREES set to 10,000. Bootstrapped parsimony analysis used 500 replicates, with one random taxon addition sequence each, TBR branch swapping, keeping up to 1000 trees per replicate, and MAXTREES set to 1000.

Five constrained analyses were performed to evaluate alternative phylogenetic hypotheses that were suggested by morphology, anatomy, and/or results of previous phylogenetic studies (see below). According to each hypothesis, a constraint was constructed in MacClade version 4.0 (Maddison and Maddison, 1997) forcing monophyly of only one node at a time without any other topological specifications. Maximum parsimony analyses were performed under the constraints, using the same settings as described for the unconstrained analysis. The resulting constrained and unconstrained trees were sorted by their likelihood scores. The calculation of the likelihood scores used the HKY85 model of sequence evolution (Hasegawa et al., 1985) implemented in PAUP* with empirical base frequencies, transition-transversion bias set to two, two substitution types, and an equal distribution of rates at variable sites. For each constraint the ten most likely unconstrained and constrained MP trees were compared using the Shimodaira and Hasegawa (S–H) test (Shimodaira and Hasegawa, 1999), as implemented in PAUP*. The S–H test used the resampling estimated log-likelihood (RELL) method with 1000 replicates.

Constraint one (*Henningsomyces–Rectipilus*) forced all samples of *Henningsomyces* and *Rectipilus* to form a monophyletic group. Based on anatomical characters, both genera are well-defined taxa and represent putatively monophyletic groups (Agerer, 1973, 1983b; Cooke, 1989; Singer, 1986). Constraint two (*Calathella*) forced monophyly of three representatives of the anatomically well-characterized cyphelloid genus *Calathella* (Agerer, 1983b; Jones and Agerer, 1992; Reid, 1964), *viz.*, *C. gayana*, *C. mangrovii*, and *C. columbiana*. Constraint three (*Nia*/schizophylloid) forced monophyly of the *Nia* clade and the /schizophylloid clade (named according to Moncalvo et al., 2002). Previous phylogenetic analyses (Binder et al., 2001; Hibbett and Binder, 2001) suggested that they could be sister groups. Constraint four (*Crepidotus* A) forced monophyly of the agaricoid genera *Crepidotus*, *Simocybe*, *Inocybe*, *Pleuroflammula*, and *Tubaria*, as well as the cyphelloid taxa *Pellidiscus* and *Phaeosolenia*. Constraint five (*Crepidotus* B) forced *Crepidotus*, *Pellidiscus*, *Phaeosolenia*, and *Simocybe* to form one clade. The latter two constraints follow the findings of Moncalvo et al. (2002) that suggested monophyly of *Crepidotus*, *Simocybe*, *Inocybe*, and *Pleuroflammula*. The putative inclusion of *Tubaria*, *Phaeosolenia*, and *Pellidiscus* was supported by anatomy based taxonomy (Singer, 1986).

Analyses of data set II used maximum parsimony and maximum likelihood (ML) with all characters included. Maximum parsimony analysis treated characters as unordered, and equally weighted. The analysis involved 1000 heuristic searches with random taxon addition sequences, TBR branch swapping, and MAXTREES set at 10,000. Bootstrapped MP analysis used 1000 replicates, with one random taxon addition sequence each, TBR branch swapping, keeping up to 1000 trees per replicate, and MAXTREES set to 1000.

Modeltest version 3.06 (Posada and Crandall, 1998) was used to identify an optimal model of sequence evolution for ML analysis of data set II, which was the

GTR + G + I model with six substitution types, nucleotide frequencies set to A = 0.28340, C = 0.18280, G = 0.25730, T = 0.27650, and the distribution of rates at variable sites modeled on a discrete gamma distribution with four rate classes and shape parameter $\alpha = 0.7139$. The ML analysis employed a heuristic search with the best trees obtained in the MP analysis used as starting trees for TBR branch swapping with MAXTREES set to 1000. Bootstrapped ML analysis used 1000 replicates with one random taxon addition sequence per replicate, TBR branch swapping, and MAXTREES set to 1000.

Three constrained analyses were performed on data set II, corresponding to the first three constrained analyses conducted on data set I. Constraints one to three forced monophyly of the representatives of *Henningsomyces* and *Rectipilus*, *Calathella*, as well as the *Nia*- and the /schizophylloid clade, respectively. Constrained analyses were performed using the same settings as the unconstrained analysis. Constrained as well as unconstrained MP trees were sorted by their likelihood scores using the HKY85 model of sequence evolution. For each constraint the 10 most likely unconstrained and constrained MP trees were compared using the S–H test with 1000 RELL replicates.

3. Results

3.1. PCR and sequencing

PCR products were obtained from 31 (ca. 40%) of the DNA's extracted from cyphelloid material. PCR product sizes of partial nuc-lsu rDNA usually ranged from ca. 0.92 to 0.97 kb. Nuc-lsu rDNA products of *Henningsomyces candidus* PB338, *H. puber* GUA-307, and *H.* spec. C58569 were ca1.1 and 1.3 kb, respectively, showing an insertion next to the 3′ end of the LR5 primer region. The alignment did not include the inserted sequences. PCR products of ITS rDNA ranged from 568 to 767 bp. In total, 27 nuc-lsu and 30 ITS rDNA sequences of cyphelloid samples were generated. Thirteen nuc-lsu and eight ITS rDNA sequences were obtained for other forms (Table 1). Cyphelloid ITS spacer regions 1 and 2 were too divergent to be alignable over the represented taxa. An 170 bp partition of the conserved 5.8S rDNA region was included in analyses of data set II.

PCR products of five samples (*Aphyllotus campanelliformis* PB306/RA, *Calathella eruciformis* PB310/RA, *Flagelloscypha kavinae* PB346/RA5505, *Maireina* spec. PB319/RA6018, and *Seticyphella niveola* PB311/RA) represented not the target samples, but rDNA from macroscopically undetectable ascomyceteous contaminants, which were identified by a BLAST search. PCR products of three cyphelloid samples corresponded to homobasidiomyceteous sequences, but were determined to be putatively erroneous. One sequence obtained from *Woldmaria crocea* HorakB1 was placed in the polyporoid clade with NJ bootstrap support of 71%, but another isolate of *W. crocea*, NH-10.23.95, was placed in a strongly supported (NJ bootstrap = 91%) group within the euagarics clade, which was dominated by cyphelloid forms. The sequence obtained from a culture of *Lachnella villosa* (CBS609.87) was nested within representatives of the non-cyphelloid genera *Auriculariopsis* and *Schizophyllum* (NJ bootstrap = 83%), but three other *Lachnella* sequences (obtained from two specimens of *L. villosa* and one of *L. alboviolascens*) formed a separate, strongly supported (NJ bootstrap = 94 %) monophyletic group. Finally, the sequence from a culture of *Stromatoscypha fimbriata* (CBS321.58) was suggested as sister group of *Pholiota lenta* (NJ bootstrap = 99%), whereas two previously generated sequences of *S. fimbriata* (AF261370, AF261371) were placed in the /hydropoid clade sensu Moncalvo et al. (2002) (NJ bootstrap = 98%) confirming the results of this previous study. In all three cases, the results of a BLAST search using the putatively erroneous sequences as query returned results correspondent to the results of the preliminary analyses. There is no anatomical evidence that the three putatively erroneous samples were correctly placed. Since misidentification of the original cyphelloid material or PCR and laboratory errors could not be ruled out those sequences were pruned from the preliminary data set before assembling data set I.

3.2. Analyses of data set I

Data set I had an aligned length of 977 characters, with 514 variable, and 379 parsimony-informative positions. Step one of the unconstrained MP analysis recovered two trees of 4704 steps. TBR branch swapping on these trees produced 9588 most parsimonious trees of 4703 steps (CI = 0.184, RI = 0.609) (Fig. 1).

In general, the higher order relationships within the euagarics clade received weak bootstrap support. Nevertheless, 74 nodes received support of at least 70%. Clades are labeled using the notation and terminology of Moncalvo et al. (2002, e.g., "/schizophylloid clade"). Cyphelloid taxa are placed in 11 different groups within the euagarics clade (Fig. 1). Seven of the 16 included cyphelloid genera are concentrated in the strongly supported (bootstrap = 95%) *Nia* clade, including samples of *Calathella*, *Cyphellopsis*, *Flagelloscypha*, *Halocyphina*, *Lachnella*, *Merismodes*, and *Woldmaria*. The *Nia* clade is named after the gasteromycete *Nia vibrissa*, which forms a well-supported *Nia* core clade of marine homobasidiomycetes (bootstrap = 84%) with the cyphelloid taxa *Halocyphina villosa* and *Calathella mangrovii*, as previously recognized by Hibbett and Binder (2001). This marine clade is placed among terrestrial cyphelloid taxa, of which *Flagelloscypha* and *Lachnella*, as well as

Fig. 1. Phylogenetic placement of cyphelloid homobasidiomycetes in the euagarics clade inferred from maximum parsimony analyses of nuc-lsu rDNA sequences. Tree 1/9588 (4703 steps, CI = 0.184, RI = 0.609). Bootstrap frequencies $\geqslant 50\%$ are shown above branches. Branches that collaps in the strict consensus tree are marked with an asterisk. Names of cyphelloid samples are given in bold. Cyphelloid samples that are not included in labeled clades are marked with an arrow. Clades that have been recognized by Moncalvo et al. (2002) are indicated by/in front of the name of the clade. Samples, of which new sequences had been generated in this study, are given with isolate number, sequences downloaded from GenBank are given with accession number.

Cyphellopsis and *Merismodes*, respectively, form weakly supported (bootstrap = 70%) monophyletic groups. The *Nia* clade also includes two corticioid forms, *Dendrothele acerina* and *D. griseocana*.

Amyloflagellula inflata is the only cyphelloid species placed in the /marasmioid clade sensu Moncalvo et al. (2002) (bootstrap = 90%). The sister group of *A. inflata* is an undescribed marasmioid species with a poroid

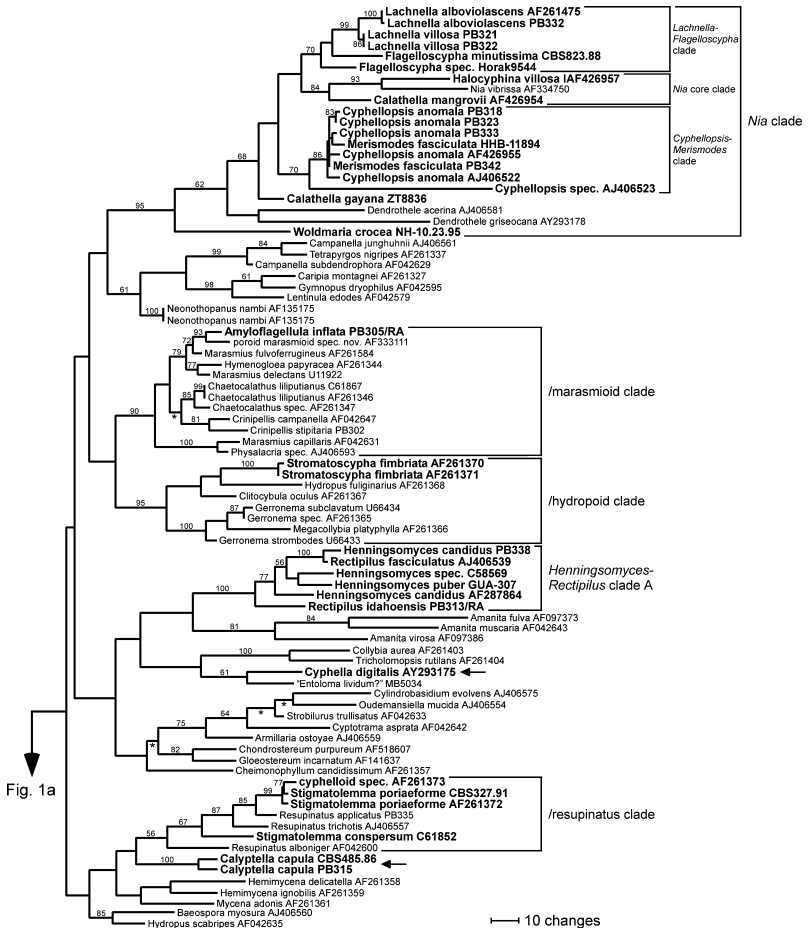

Fig 1. (*continued*)

hymenophore (bootstrap = 93%). The /marasmioid clade also includes two well-supported subclades (bootstrap = 85 and 81%, respectively) that contain representatives of the gilled genera *Chaetocalathus* and *Crinipellis*, which Singer (1986) suggested are closely related.

The present study, included six samples of the cyphelloid genus *Henningsomyces*, and three samples of the morphologically and anatomically similar genus *Rectipilus*. They were placed in two strongly supported clades: *Henningsomyces–Rectipilus* clade A (bootstrap = 100%), which contains four samples of *Henningsomyces* and two of *Rectipilus*, and *Henningsomyces–Rectipilus* clade B (bootstrap = 99%), which contains two samples of *Henningsomyces* and one of *Rectipilus*. *Henningsomyces–Rectipilus* clades A and B are not closely related in the MP tree (Fig. 1).

The cyphelloid genus *Stigmatolemma* is nested in the weakly supported (bootstrap = 56%) /resupinatus clade sensu Moncalvo et al. (2002), which includes three species of the gilled genus *Resupinatus* as well as three identified samples of *Stigmatolemma*. An unidentified cyphelloid sample forms a strongly supported (bootstrap = 99%) monophyletic group with two samples of *S. poriaeforme* and putatively represents a further *Stigmatolemma* specimen. The cyphelloid species *Calyptella capula* is placed as the sister group of the /resupinatus clade, but without bootstrap support.

The type species of the "Cyphellaceae," *Cyphella digitalis*, is weakly supported (bootstrap = 61%) as the sister group of a sequence that was thought to represent the agaric *Entoloma lividum* (Fig. 1). However, this sequence did not cluster with the four other sequences of *Entoloma* in the data set, suggesting that the "*E. lividum*" sequence may be misidentified or a contaminant.

The placements of the remaining cyphelloid samples in the MP trees received less than 50% bootstrap support. The cyphelloid species *Pellidiscus pallidus* is nested in the /crepidotoid clade sensu Moncalvo et al. (2002), which is represented here by three *Crepidotus* and one *Simocybe* species. *Calathella columbiana* is placed as sister group of *Mycenoporella lutea*, which is a mycenoid fungus with poroid hymenophore. Two other species of *Calathella*, *C. mangrovii* and *C. gayana*, are nested in the *Nia* clade, however. *Phaeosolenia densa* is placed as a sister group of the collybioid *Tubaria hiemalis*, which represent the /tubaria clade sensu Moncalvo et al. (2002). Two samples of the cyphelloid species *S.*

Table 2
Comparison of unconstrained and constrained MP trees obtained with data sets I and II

	No. of trees	Steps	CI	RI	$-\ln L$	S–H test
Data set I						
Unconstrained MP analysis	9588	4703	0.184	0.609	28212.02558	$P=0.481$
Constraint 1 [*Henningsomyces–Rectipilus*]	10,000	4719	0.184	0.607	28244.07226	$P=0.258$
Constraint 2 [*Calathella*]	10,000	4759	0.182	0.603	28378.41416	$P=0.008^*$
Constraint 3 [*Nia*-/schizophylloid]	10,000	4705	0.184	0.609	28224.53709	$P=0.411$
Constraint 4 [*Crepidotus* A]	10,000	4707	0.184	0.608	28264.08822	$P=0.206$
Constraint 5 [*Crepidotus* B]	10,000	4711	0.184	0.608	28207.51489	Best
Data set II						
Constraint 1 [*Henningsomyces–Rectipilus*]	36	983	0.479	0.751	7098.91242	$P=0.487$
Constraint 2 [*Calathella*]	108	1031	0.457	0.727	7284.18791	$P=0.000^*$
Constraint 3 [*Nia*-/schizophylloid]	69	977	0.482	0.754	7089.23642	$P=0.779$

The best tree according to likelihood score is provided for unconstrained and each case of constrained MP analyses performed on data sets I and II. Constrained trees that can be rejected based on the results of the S–H test ($P<0.050$) are indicated by an asterisk.

fimbriata are placed in the /hydropoid clade forming the sister group of *Hydropus fuliginarius*.

The weakly supported (bootstrap = 55%) /schizophylloid clade includes no cyphelloid species sensu stricto. It is formed by two strongly supported (bootstrap = 100%) sister groups containing *Schizophyllum* and *Auriculariopsis*, and *Fistulina* and *Porodisculus*, respectively.

The phylogenetic topology suggested by unconstrained MP trees was compared with trees obtained in constrained analyses performed on data set I. The results of the S–H test (Table 2) indicate that only in the case of constraint two (*Calathella*) can the constrained trees be rejected, suggesting polyphyly of the cyphelloid genus *Calathella*. In the case of constraints one (*Henningsomyces–Rectipilus*), three (*Nia*-/schizophylloid), and four (*Crepidotus* A), none of the constrained trees can be rejected. In the case of constraint five (*Crepidotus* B), the S–H test indicates that the constrained trees have a better likelihood score than the unconstrained trees.

3.3. Analyses of data set II

Data set II had an aligned length of 1082 characters, with 350 variable, and 270 parsimony-informative positions. The cyphelloid taxa *C. digitalis* and *S. fimbriata*, and seven species of agarics that were placed alongside cyphelloid forms in the /crepidotoid clade, /tubaria clade, and /resupinatus clade were not included in data set II, because they lack 5.8S rDNA sequences. Maximum parsimony analysis produced 81 trees (975 steps, CI = 0.483, RI = 0.755). The groupings of cyphelloid and non-cyphelloid taxa in trees derived from data set II are consistent with the groupings obtained in analyses of data set I (Figs. 1, 2). Levels of bootstrap support for the individual clades containing cyphelloid forms in analyses of data set II are equal to or higher than those in analyses of data set I, except for *L. villosa* and the *Nia* core clade, which received slightly stronger support in analyses of data set I (Figs. 1, 2). The backbone of the phylogeny is almost completely unresolved, however (Fig. 2).

The tree recovered by ML analysis of data set II ($-\ln L = 6472.46293$) is consistent with the strict consensus of the MP trees (Figs. 2 and 3). *P. pallidus* and *P. densa* form a monophyletic group that was not resolved in the strict consensus of the MP trees, but that was present in 18 of the 81 most parsimonious trees. This clade received less than 50% bootstrap support, however. Additionally, the ML tree suggests that *C. capula* is the sister group of the /resupinatus clade, but with weak bootstrap support. Both the ML tree and the strict consensus of the MP trees suggest that the *Henningsomyces–Rectipilus* clade A is the sister group of the *Nia* clade (but without bootstrap support), and is not closely related to the *Henningsomyces–Rectipilus* clade B (Figs. 2 and 3).

Constrained analyses one to three were performed on data set II corresponding to the phylogenetic hypotheses tested on data set I and compared to unconstrained trees (Table 2). Results of the S–H test suggest that only in the case of constraint two (*Calathella*) can the constrained trees be rejected, whereas none of the trees retrieved under constraints one and three (*Henningsomyces–Rectipilus* and *Nia*-/schizophylloid) can be rejected.

4. Discussion

Results of the present study confirm that cyphelloid homobasidiomycetes are a polyphyletic group of species that have been derived by reduction from within the euagarics clade (Agerer, 1978a; Donk, 1959; Horak and Desjardin, 1994; Singer, 1966, 1986). Unconstrained topologies suggest that there have been about 10–12 independent origins of cyphelloid forms, although

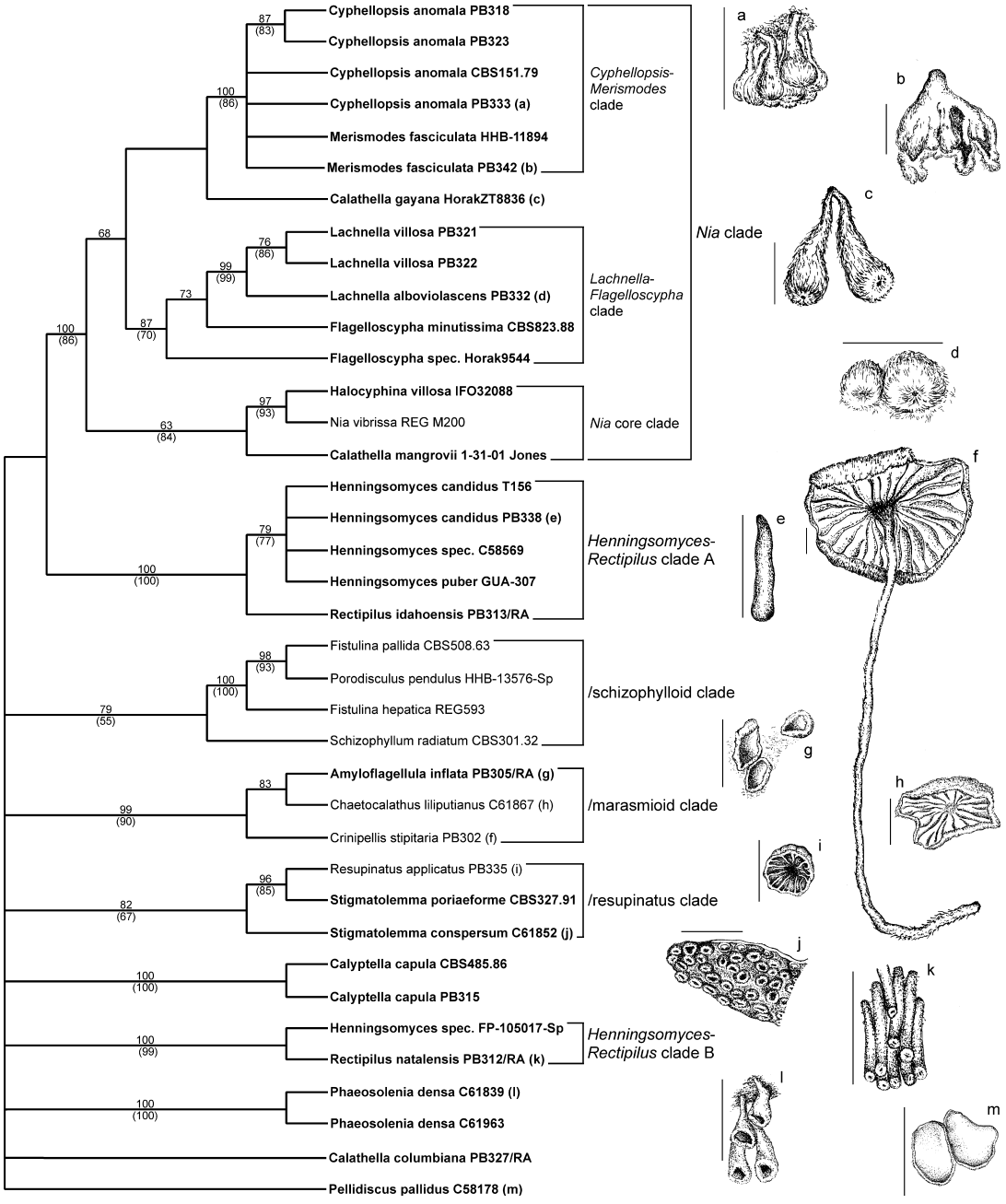

Fig. 2. Phylogenetic relationships of cyphelloid homobasidiomycetes inferred from maximum parsimony analysis of nuc-lsu rDNA and 5.8S rDNA sequences. Strict consensus of 81 most parsimonious trees. Bootstrap frequencies $\geqslant 50\%$ are shown above branches, corresponding bootstrap values from analyses of data set I are given in parentheses below branches. Clades are labeled according to Fig. 1. Names of cyphelloid samples are given in bold. The fruiting body habit of representatives of selected taxa is illustrated: (a) *Cyphellopsis anomala*; (b) *Merismodes fasciculata*; (c) *Calathella gayana*; (d) *Lachnella alboviolascens*; (e) *Henningsomyces candidus*; (f) *Crinipellis stipitaria*; (g) *Amyloflagellula inflata*; (h) *Chaetocalathus liliputianus*; (i) *Resupinatus applicatus*; (j) *Stigmatolemma conspersum*; (k) *Rectipilus natalensis*; (l) *Phaeosolenia densa*; and (m) *Pellidiscus pallidus*. Scale bar = 1 mm. Corresponding letters are given in parentheses next to illustrated samples.

evaluation of constrained topologies indicates that there may have been fewer origins. In the following sections, the phylogenetic groupings of cyphelloid taxa are discussed based on both molecular and non-molecular evidence. The questionable phylogenetic placements of the non-cyphelloid species *Calocybe persicolor*, *Megacollybia platyphylla*, and *Rozites caperatus*, which are in conflict with previous results and/or anatomical evidence, are not considered within the scope of this discussion.

The *Nia* clade represents the major concentration of cyphelloid forms in the euagarics clade. Previous studies revealed that the cyphelloid taxa *H. villosa*, *C. mangrovii*, and *Cyphellopsis anomala* as well as the marine gasteromycete *N. vibrissa* are in this group (Hibbett and

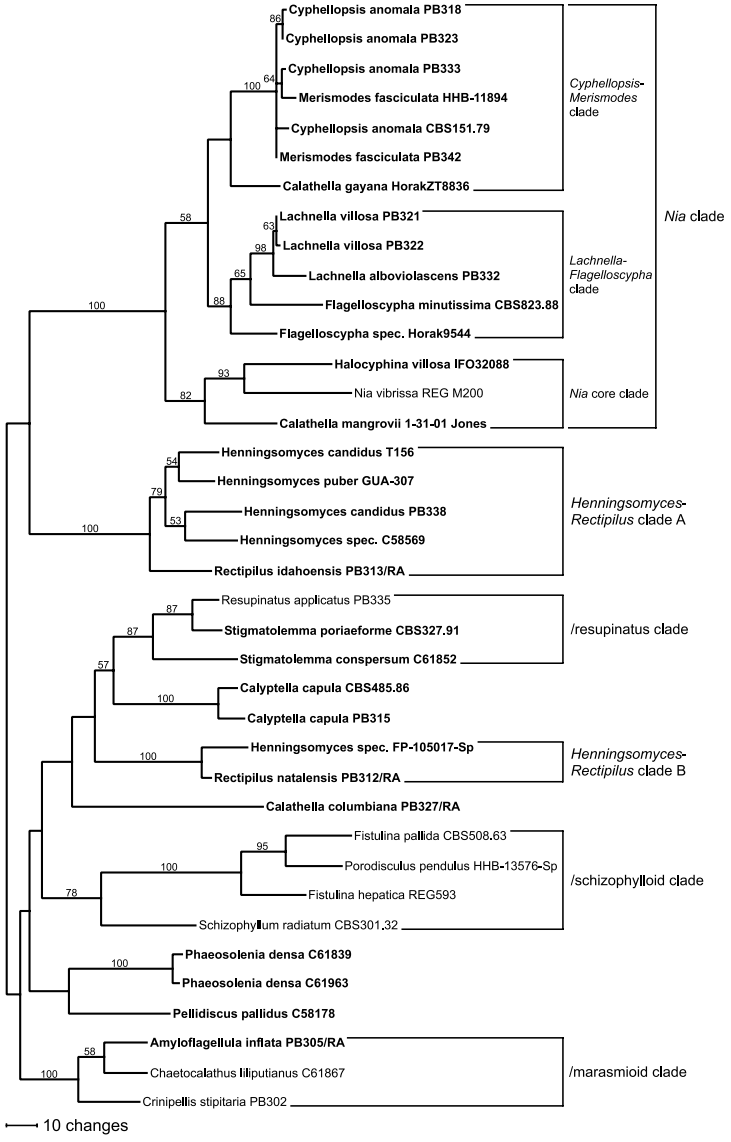

Fig. 3. Phylogenetic relationships of cyphelloid homobasidiomycetes inferred from maximum likelihood analysis (tree score: $-\ln L = 6472.46293$) from nuc-lsu rDNA and 5.8S rDNA sequences. Maximum likelihood bootstrap frequencies $\geqslant 50\%$ are shown above branches. Names of cyphelloid samples are given in bold. Clades are labeled according to Figs. 1 and 2.

Binder, 2001). The present study shows that the cyphelloid species *Calathella gayana*, *C. anomala*, *Flagelloscypha minutissima*, *Lachnella alboviolascens*, *L. villosa*, *Merismodes fasciculata*, and *W. crocea* also belong to this clade. Four of the included genera (*Calathella*, *Cyphellopsis*, *Merismodes*, and *Woldmaria*) were previously classified in the Cyphellopsidaceae (Jülich, 1982), which the present results suggest is not monophyletic.

Present findings agree with those of previous molecular studies that suggested close relationships between aquatic homobasidiomycetes represented by the *Nia* core clade and terrestrial cyphelloid taxa (Binder et al., 2001; Hibbett and Binder, 2001). The *Nia* core clade contains three marine species, *N. vibrissa*, which grows on fully submerged substrates, as well as the mangrove-inhabiting cyphelloid species *H. villosa* and *C. mangrovii* (Hibbett and Binder, 2001). All other members of the *Nia* clade are purely terrestrial, which indicates that the marine habit is derived in this group. *N. vibrissa* represents a unique case of derivation of a gasteroid form from cyphelloid precursors.

Monophyletic groups are formed by *Lachnella* plus *Flagelloscypha*, as well as *Cyphellopsis* plus *Merismodes*. These relationships had already been suggested by anatomical evidence. *Lachnella* and *Flagelloscypha* share major characters of basidia, spores, and surface hyphae (Agerer, 1983b; Reid, 1964; Singer, 1986). The delimitation of these genera is mainly based on the different shape of the distal part of the surface hyphae, *viz* tapering, whip-like ends that lack a crystal covering in *Flagel-*

loscypha, vs. non-tapering and completely encrusted tips in *Lachnella* (Agerer, 1975, 1979b,c, 1980a, 1983b, 2002; Reid, 1964). These two genera are linked by species with intermediate pattern of characters (Agerer, 1979d, 1983b) and have been placed in the Lachnellaceae (Agerer, 1983b; Jülich, 1982). Results of the present study suggest that *Flagelloscypha* is paraphyletic (Figs. 1–3).

Cyphellopsis and *Merismodes* are distinguished by the habit of their fruiting bodies. *Merismodes* produces fascicle-like complexes of tightly connate fruiting bodies (Fig. 2), whereas *Cyphellopsis* has single to branched or proliferating compound fruiting bodies that are seated in a more or less well-developed subiculum (Reid, 1964) (Fig. 2). Data sets I and II included multiple samples of *M. fasciculata* and *C. anomala*, the latter representing a species complex of anatomically similar taxa. Consistent with anatomical evidence, results of both MP and ML analyses suggest that *Cyphellopsis* and *Merismodes* are very closely related (Figs. 1–3). The internal relationships of the *Cyphellopsis–Merismodes* clade could not be resolved with data sets I or II. The separation of *Cyphellopsis* and *Merismodes* based on morphological and anatomical characters has been controversial (Agerer, 1975, 1978b, 1980b, 1983b; Agerer et al., 1980; Cooke, 1962, 1989; Donk, 1931, 1959, 1962; Kirk et al., 2001; Moser, 1983; Reid, 1961, 1964; Singer, 1986). The results of the present study do not support recognition of two separate genera (Figs. 1–3).

The *Nia* clade also contains two corticioid species, *D. acerina* and *D. griseocana*. A relationship of *D. acerina* with the cyphelloid taxa *L. villosa* and *C. anomala* (as well as to *Schizophyllum commune*) was suggested by a previous molecular phylogenetic analysis (Langer, 2002). Main parts of the hymenia of the *Dendrothele* species are formed by irregularly branched hyphal elements with a dense crystal covering (Eriksson and Ryvarden, 1975), which resemble the surface hairs found in some cyphelloid taxa (Agerer, 1978b, 1983a,b; Horak and Desjardin, 1994; Reid, 1961; Singer, 1986). The cyphelloid species in the *Nia* clade have non-ramified surface hyphae (Agerer, 1975, 1978b, 1986b; Cooke, 1962; Reid, 1964), however. There is no obvious anatomical similarity of corticioid and cyphelloid forms in the *Nia* clade.

Analyses of data set I suggest that *W. crocea* is placed as the sister group to the other members of the *Nia* clade (Fig. 1). This relationship is noteworthy in that it is the only cyphelloid species in the *Nia* clade that has surface hyphae without a crystal covering and differs from other cyphelloid forms in having sigmoid to fusiform spores and the habit of growing specifically on the ostrich fern *Matteuccia struthiopteris* (Agerer, 1983b; Cooke, 1962; Woldmar, 1954).

Three samples of the cyphelloid genus *Calathella* were included in data sets I and II. In all analyses, the marine species *C. mangrovii* and the terrestrial *C. gayana* are placed in the *Nia* clade, whereas *C. columbiana* is placed separately (Figs. 1–3). Analyses of data set I suggest that *C. columbiana* is the sister group of *M. lutea*, a mycenoid species with a poroid hymenophore (Horak, 1968; Singer, 1986). Results of the S–H tests on constrained topologies suggest that monophyly of *Calathella* can be rejected (Table 2). Nevertheless, the genus *Calathella* is anatomically well-characterized by distinctly suburniform basidia with widened middle, cylindric to allantoid spores, and completely encrusted surface hyphae (Agerer, 1983b). Additionally, all *Calathella* species produce proliferating or to a various degree connate fruiting bodies (Agerer, 1983b; Bodensteiner et al., 2001). Considering this combination of distinctive characters, the separation of *C. columbiana* from other *Calathella* species is surprising. Besides the taxa included here, there are five other described species of *Calathella* (Agerer, 1983b; Bodensteiner et al., 2001; Jones and Agerer, 1992; Reid, 1964). Inclusion of sequences of these taxa and additional samples of *C. columbiana* are required to fully resolve the status of *Calathella.*

Previous phylogenetic studies (Binder et al., 2001; Hibbett and Binder, 2001; Langer, 2002) suggested that the /schizophylloid clade could form the sister group of the *Nia* clade. With *Schizophyllum* and *Fistulina* the /schizophylloid clade contains genera, whose unique fruiting bodies with "split gills" in case of *Schizophyllum* and a "pore-like" hymenophore composed of individual tubes in the case of *Fistulina* resemble aggregates of single cyphelloid fruiting bodies (Cooke, 1989; Donk, 1964; Nuss, 1980; Singer, 1986). The putative relationship of *Schizophyllum* and *Fistulina* with cyphelloid taxa is controversial and has been discussed by different authors (Agerer, 1978a; Bondarzew and Singer, 1941; Cooke, 1962; Donk, 1959, 1964; Lohwag and Follner, 1936; Singer, 1986). The putative sister group relationship of the *Nia* clade and the /schizophylloid clade was not revealed by MP and ML analyses of the current study. However, results of constrained analyses on data sets I and II indicate that the hypothesis that these clades form a monophyletic group can not be rejected (Table 2).

One of the cyphelloid taxa that has been suggested to be related to *Schizophyllum* is the genus *Stromatoscypha* (syn. *Porotheleum)*, which produces aggregates of fruiting bodies that are densely crowded on a membranous subiculum (Cooke, 1989; Donk, 1951, 1959; Reid, 1964). *Stromatoscypha* has been classified in the Schizophyllaceae by Donk (1964), but analyses of data set I suggest that *S. fimbriata* is placed in the /hydropoid clade (Fig. 1), which is consistent with previous findings by Moncalvo et al. (2002). Monophyly of the /hydropoid clade is strongly supported (bootstrap = 95%), but the precise placement of *S. fimbriata* within this group is not resolved with confidence. In the MP trees, it is the sister group of *H. fuliginarius*, which is a pileate,

gilled fungus with laticiferous hyphae and amyloid spores (i.e., staining blue in iodine). None of these characters are found in *S. fimbriata* (Donk, 1951, 1959; Kühner, 1938; Reid, 1964; Singer, 1986). Therefore, analyses of additional species of the /hydropoid clade is required to infer morphological evolution in the group, and to resolve the phylogenetic placement of *S. fimbriata*.

Both MP and ML trees suggest that there are two separate clades, each strongly supported, including samples of the genera *Henningsomyces* and *Rectipilus*, although monophyly of these two clades could not be rejected by the S–H test (Table 2). The identification of two separate *Henningsomyces–Rectipilus* clades A and B could not have been easily predicted by anatomical evidence. For both genera, different putative relationships had been suggested previously, with the Lachnellaceae including *Lachnella* and *Flagelloscypha* in case of *Rectipilus* (Agerer, 1983b), with the Schizophyllaceae (Donk, 1964) or with *Calyptella* (Agerer, 1983b) in the case of *Henningsomyces*.

Representatives of *Henningsomyces* and *Rectipilus* produce very similar looking, more or less tubular fruiting bodies (Fig. 2), whose micro-characters, such as spore shape and size, differ only slightly (Agerer, 1973, 1979a, 1983b, 1986a; Cooke, 1989; Singer, 1986; Vila et al., 1999). Unlike *Rectipilus* species, *Henningsomyces* species produce slightly gelatinous fruiting bodies (Agerer, 1973, 1983b). However, the delimitation of the two genera is mainly based on the branching pattern of the surface hyphae, which is considered a taxonomically important character at the generic level among cyphelloid fungi (Agerer, 1973, 1980b, 1982, 1983a). All members of *Henningsomyces* produce consistently branched surface hyphae, whereas those of *Rectipilus* species are usually non-ramified (Agerer, 1973, 1979a, 1983b). Results of the current study suggest independent transitions in the branching mode within the *Henningsomyces–Rectipilus* clades, and call the validity of the present delimitation and species composition of these genera into question. Neither the geographical origins of the included samples (Table 1) nor any obvious anatomical characters distinguish the recognized clades A and B. To evaluate the significance of the branching pattern of the surface hyphae as a distinctive taxonomic feature, the inclusion of multiple samples representing other *Henningsomyces* and *Rectipilus* species is required.

Maximum parsimony trees obtained with data sets I and II suggest that the cyphelloid species *A. inflata* is placed among marasmioid taxa, all of which share the presence of dextrinoid (i.e., staining reddish brown in iodine solution) hyphae or parts of hyphae (Agerer, 1978a; Agerer and Boidin, 1981; Singer, 1942, 1966, 1976, 1986). The species that represent this clade in data set II possess dextrinoid hairs, which cover the fruiting bodies of *Crinipellis stipitaria* and *Chaetocalathus liliputianus*, or dextrinoid whip-like appendices of hyphae that form the subiculum and the external cuticle of the fruiting bodies of *A. inflata* (Agerer, 1978a; Agerer and Boidin, 1981; Singer, 1942, 1986). *A. inflata* produces cup-shaped fruiting bodies with a smooth hymenophore (Agerer and Boidin, 1981; see also Fig. 2). It is a typical cyphelloid representative of the genus *Amyloflagellula*, which includes both cyphelloid and agaricoid species (Agerer and Boidin, 1981), whereas the genera *Crinipellis* and *Chaetocalathus* have gilled fruiting bodies (Fig. 2). Based on the anatomical similarities, these three genera have been placed in one tribe by Singer (1986). Putative relationships with *Crinipellis* and *Chaetocalathus* also have been suggested for the cyphelloid genera *Lachnella* and *Flagelloscypha* (Agerer, 1978a, 1983b; Donk, 1959; Singer, 1986), which include species with dextrinoid surface hyphae. Results of the present study, however, suggest that *Lachnella* and *Flagelloscypha* are placed in the *Nia* clade (Figs. 1–3).

The relationship of the cyphelloid genus *Stigmatolemma* with the gilled genus *Resupinatus* agrees with expectations based on anatomy as well as with previous results of molecular studies by Moncalvo et al. (2002). Members of both genera produce more or less cupulate, gelatinous fruiting bodies, whose external cuticle is formed by irregularly diverticulate hyphae. They also share characters such as the possession of pigmented hyphae and characteristic globule bearing structures in different parts of the fruiting bodies, as well as major spore features (Agerer, 1978a,b; Cooke, 1962; Donk, 1962; Singer, 1986; Thorn and Barron, 1986). Additionally, there are similarities in the way of living, *viz.*, the putative parasitism of members of both genera (Thorn and Barron, 1986).

Stigmatolemma and *Resupinatus* differ in the structure of the hymenophore and the arrangement of the fruiting bodies. *Stigmatolemma* species possess a permanently smooth hymenium, whereas in *Resupinatus* fruiting bodies often have an almost smooth hymenophore in early stages, but consistently produce gills with age. Fruiting bodies of *Resupinatus* species are solitary or grow in scattered groups, whereas *Stigmatolemma* species have a tendency to form compound aggregates of mostly densely crowded single fruiting bodies that are connected by a system of basal hyphae (a thin subiculum in case of *S. poriaeforme*, a stipitate, compact structure in case of *S. conspersum*) (Agerer, 1978a,b; Cooke, 1962; Donk, 1962). Based on close anatomical similarities to *Resupinatus* and *Stigmatolemma* (Agerer, unpubl., Singer, 1986; Thorn and Barron, 1986) it can be expected that the cyphelloid genera *Aphyllotus*, *Stromatocyphella*, and *Rhodocyphella* might also be placed in the /resupinatus clade.

Results of MP and ML analyses on data set I and II (Figs. 1, 3) suggest that the cyphelloid species *C. capula* (the type species of *Calyptella*) is the sister group of the /resupinatus clade, but this placement received weak

bootstrap support. *C. capula* produces cupulate fruiting bodies covered by surface hyphae with multiply branched, coralloid excrescences that lack a crystal covering (Cooke, 1962; Donk, 1951; Reid, 1961; Singer, 1962, 1986). Based on anatomy, putative relationships with cyphelloid genera like *Cyphella* (Singer, 1986) or *Henningsomyces* (Agerer, 1983b) have been suggested. The results of Moncalvo et al. (2002) indicated that *C. capula* is placed in the /hemimycena clade as the sister group of *Hemimycena ignobilis*, a delicate agaricoid species with well-developed gills (Moser, 1983; Singer, 1986). Typical *Calyptella* species and members of the /resupinatus clade share a similar general branching pattern of the surface hyphae. The, slight (at most) gelatinosity of the fruiting bodies (Singer, 1986) and lack of globule bearing structures distinguishes *Calyptella* from *Stigmatolemma* and *Resupinatus*, however. The inclusion of additional *Calyptella* species is required in order to evaluate these characters and resolve its relationships with other cyphelloid and non-cyphelloid forms.

Pellidiscus and *Phaeosolenia* were the only cyphelloid forms included in this study that have pigmented spores. Additional similarities comprise spore shape and spore wall structure (Singer, 1986). Both taxa also show morphological and anatomical differences, however. *P. pallidus* has delicate discoid fruiting bodies (Fig. 2) with colorless surface hyphae without a crystal covering, whereas *P. densa* produces tubular to pitcher-like fruiting bodies with densely encrusted, brown surface hyphae on a stroma-like subiculum (Fig. 2). Above all, features such as spore surface and hyphal system differ in *Pellidiscus* and *Phaeosolenia* (Agerer, 1983b; Cooke, 1962; Donk, 1959, 1962; Moser, 1983; Reid, 1964; Singer, 1986).

Maximum parsimony analysis of data set I suggests that *P. pallidus* is placed in the /crepidotoid clade sensu Moncalvo et al. (2002) along with the agaric genera *Crepidotus* and *Simocybe*, and that *P. densa* is placed in the /tubaria clade along with the agaric genus *Tubaria*, but both groups received no bootstrap support (Fig. 1). Based on spore characters, *Pellidiscus* and *Phaeosolenia* have been suggested to be related to agaricoid genera typically placed in the Crepidotaceae, which are characterized by yellowish to brownish, non-angular spores (Cooke, 1962; Donk, 1959, 1962; Reid, 1964; Singer, 1962, 1986). Additionally, the results of Moncalvo et al. (2002) indicated that the genera *Inocybe* and *Pleuroflammula* may form a monophyletic group with the /crepidotoid clade. Considering these findings, constrained analyses four (*Crepidotus* A; forcing monophyly of *Crepidotus*, *Simocybe*, *Pellidiscus*, *Phaeosolenia*, *Tubaria*, *Inocybe*, and *Pleuroflammula*) and five (*Crepidotus* B; forcing monophyly of *Crepidotus*, *Simocybe*, *Pellidiscus*, and *Phaeosolenia*) were performed on data set I. Based on the results of the S–H test, tree topologies obtained under these two constraints could not be rejected. Moreover, the S–H test suggests that topologies produced under constraint five have better likelihood scores than unconstrained trees.

Data set II included sequences of *Pellidiscus* and *Phaeosolenia*, but not *Crepidotus*, *Simocybe*, or *Tubaria*. Eighteen of 81 most parsimonious trees (not shown) as well as the ML tree (Fig. 3) suggest that *Pellidiscus* and *Phaeosolenia* form a monophyletic group, but this clade received no bootstrap support. Although their relationships could not be resolved with confidence from either data set I or II, the placement of both *Pellidiscus* and *Phaeosolenia* in the /crepidotoid clade is likely, taking into account the combined MP and ML results.

The phylogenetic placement of *C. digitalis*, which is the only accepted species in the genus *Cyphella* (Singer, 1986), remains unresolved. Based on anatomical characters, Singer (1986) suggested a putative relationship of *C. digitalis* with several other reduced taxa, among which only the cyphelloid genus *Calyptella* was included in the current study. Findings of a previous study by Hibbett and Binder (2002) including nuc-lsu rDNA and mt-ssu rDNA regions of *C. digitalis* suggested that this species is not even placed in the euagarics clade but in the polyporoid clade, another of the eight major clades of homobasidiomycetes recognized by Hibbett and Thorn (2001). To resolve the relationships of this problematic species, it will be necessary to study additional gene loci in multiple samples.

In summary, results of the present study indicate that cyphelloid forms are a polyphyletic assemblage of taxa in the euagarics clade. Unconstrained topologies suggest that there have been at least 10–12 independent origins of cyphelloid forms from agaricoid ancestors. Taking the results of constrained analyses into account, however, there may have been as few as 8–9 origins of cyphelloid forms. Whatever their number, the origins of cyphelloid forms represent striking cases of parallel evolutionary reduction. Among the known cyphelloid taxa, there are approximately 24 genera and 95 species that are not represented in the present data sets. As these taxa are added, additional origins of these fungi with minute, cup-shaped fruiting bodies may be discovered.

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