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The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods

Jean-Marc Moncalvo[1]
Department of Natural History, Royal Ontario Museum, and Department of Botany, University of Toronto, Toronto, Ontario, M5S 2C6 Canada

R. Henrik Nilsson
Göteborg University, Department of Plant and Environmental Sciences, Box 461, 405 30 Göteborg, Sweden

Brenda Koster
Department of Botany, University of Toronto, Toronto, Ontario, M5S 3B2 Canada

Susie M. Dunham
Department of Botany and Plant Pathology, Oregon State University, 2082 Cordley Hall, Corvallis, Oregon 97331

Torsten Bernauer
Universität Kassel, FB 18 Naturwissenschaften, FG Ökologie, Heinrich-Plett-Straße 40, DE-34132, Kassel, Germany

P. Brandon Matheny
Biology Department, Clark University, 950 Main Street, Worcester, Massachusetts 01610

Teresita M. Porter
Department of Botany, University of Toronto, Toronto, Ontario, M5S 3B2 Canada

Simona Margaritescu
Royal Ontario Museum, Toronto, Ontario, M5S 2C6 Canada

Michael Weiß
Sigisfredo Garnica
Spezielle Botanik und Mykologie, Universität Tübingen, Auf der Morgenstelle 1, D-72076, Tübingen, Germany

Eric Danell
Museum of Evolution, Botany Section, Uppsala University, Norbyv. 16, SE-75236, Uppsala, Sweden

Gitta Langer
Ewald Langer
Universität Kassel, FB 18 Naturwissenschaften, FG Ökologie, Heinrich-Plett-Straße 40, DE-34132, Kassel, Germany

Ellen Larsson
Karl-Henrik Larsson
Göteborg University, Department of Plant and Environmental Sciences, Box 461, SE-40530, Göteborg, Sweden

Rytas Vilgalys
Department of Biology, Box 90338, Duke University, Durham, North Carolina 27708

Abstract: We reassessed the circumscription of the cantharelloid clade and identified monophyletic groups by using nLSU, nSSU, mtSSU and RPB2 sequence data. Results agreed with earlier studies that placed the genera *Cantharellus, Craterellus, Hydnum, Clavulina, Membranomyces, Multiclavula, Sistotrema, Botryobasidium* and the family Ceratobasidiaceae in that clade. Phylogenetic analyses support monophyly of all genera except *Sistotrema*, which was highly polyphyletic. Strongly supported monophyletic groups were: (i) *Cantharellus-Craterellus, Hydnum,* and the *Sistotrema confluens* group; (ii) *Clavulina-Membranomyces* and the *S. brinkmannii-oblongisporum* group, with *Multiclavula* being possibly sister of that clade; (iii) the *Sistotrema eximum-octosporum* group; (iv) *Sistotrema adnatum* and *S. coronilla.* Positions of *Sistotrema raduloides* and *S. athelioides* were unresolved, as were basal relationships. *Botryobasidium* was well supported as the sister taxon of all the above taxa, while Ceratobasidiaceae was the most basal lineage. The relationship between *Tulasnella* and members of the cantharelloid clade will require further scrutiny, although there is cumulative evidence that they are probably sister groups. The rates of molecular evolution of both the large and small nuclear ribosomal RNA genes (nuc-rDNA) are much higher in *Cantharellus, Craterellus* and *Tulasnella* than in the other cantharelloid taxa, and analyses of nuc-rDNA sequences strongly placed *Tulasnella* close to *Cantharellus-Craterellus.* In contrast analyses with RPB2 and mtSSU sequences placed *Tulasnella* at the base of the cantharelloid clade. Our attempt to reconstruct a ''supertree'' from tree topologies resulting from separate analyses that avoided phylogenetic reconstruction problems associated with missing data and/or unalignable sequences proved unsuccessful.

Key words: Basidiomycota, Fungi, mtSSU, nLSU, nSSU, phylogeny, RPB2

INTRODUCTION

The cantharelloid clade first was first recognized by Hibbett and Thorn (2001) to accommodate a mor-

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[1] Corresponding author. E-mail: jeanmarcm@gmail.com

phologically diverse group of fungi that consistently clustered with the chanterelles (*Cantharellus* L.: Fr.) in molecular phylogenetic analyses. As presently recognized the cantharelloid clade comprises about 300 known species, making it a much smaller clade than most of the other major basidiomycete lineages (Hibbett and Thorn 2001, Binder et al 2005). *Cantharellus* was set apart from the other gilled fungi early in the history of mycology (Fries 1821) on the basis that its members form ''false'' gills resulting from a plicate hymenophore rather than developing ''true'' gills like most other mushrooms. *Craterellus* was created by Persoon (1825) to distinguish from *Cantharellus* those chanterelles having a hollow stipe, but the distinction between these two genera has long been controversial (Corner 1966, Petersen 1971). *Gomphus* Pers.: Fr. is another genus with a similarly plicate hymenial surface that traditionally was classified in the vicinity of *Cantharellus* in the order Cantharellales. *Hydnum* L.: Fr. is a genus with striking morphological, ecological and culinary similarities to the chanterelles except for having a spinose rather than a lamellate hymenophore, and most authors also classified it in the Cantharellales. Over the years the circumscription and the composition of the order Cantharellales has been much in flux. While sometimes restricted to the taxa mentioned above, the Cantharellales was also a place-holder for a multitude of aphyllophoroid genera as diverse as the toothed fungi *Auriscalpium* and *Sarcodon*, the clavarioid and coralloid genera *Clavaria, Clavariadelphus, Clavulina, Clavulinopsis, Multiclavula, Typhula, Pterula* and *Ramaria*, the cauliflower genus *Sparassis,* and poroid *Albatrellus* (Donk 1964).

Hibbett et al (1997) were the first to use DNA sequencing and phylogenetic principles for inferring evolutionary relationships from a broad taxonomic sampling of homobasidiomycetes. These authors used sequence data from both the nuclear (nSSU) and mitochondrial (mtSSU) small ribosomal subunit RNA genes that indicated a common origin of *Cantharellus, Hydnum, Clavulina*, *Multiclavula* and members of the corticioid genus *Botryobasidium*, while placing *Gomphus, Clavaria* and several other putative members of the Cantharellales in separate clades. Subsequent molecular phylogenetic studies indicated that the resupinate taxa *Sistotrema, Membranomyces* and the Ceratobasidiaceae were also members of the cantharelloid clade (Pine et al 1999, Hibbett et al 2000, Hibbett and Donoghue 2001, Hibbett and Binder 2002, Binder and Hibbett 2002, Larsson et al 2004, Binder et al 2005).

Hibbett and Thorn (2001) proposed the inclusion of the traditional heterobasidiomycete genus *Tulasnella* in the cantharelloid clade based on a mtLSU phylogeny in Bruns et al (1998) and unpublished mtSSU data. In the most recent and most comprehensive phylogenetic study of the homobasidiomycetes, Binder et al (2005) used a four-gene dataset comprising nSSU, mtSSU, nLSU and mtLSU sequences that placed *Tulasnella* as a sister group of all the other cantharelloid taxa. That study also indicated that the Sebacinales could be included in the cantharelloid clade.

All studies to date that included members of the cantharelloid clade were either within a much broader basidiomycete framework (as referred above) or restricted to genus-level investigations (Dahlman et al 2000, Dunham et al 2003, Thacker and Henkel 2004, Henkel et al 2005). Studies relying on nSSU and/or nLSU sequences of *Cantharellus, Craterellus* and/or *Tulasnella* for inference of intergeneric phylogenetic relationships have been plagued with alignment difficulties due to an accelerated rate of molecular evolution of the nuclear rDNA genes in these taxa, resulting in their placement on distinctively long branches. Moreover most earlier studies used parsimony or distance-based reconstruction methods that are known to be more sensitive to the long-branch attraction problem than likelihood-based methods and therefore can result in misleading inference of evolutionary relationships (Felsenstein 1978, Huelsenbeck 1997, Cunningham et al 1998, Poe and Swofford 1999). A reassessment of the cantharelloid clade in a more focused taxonomic context therefore is warranted.

The aim of the present study was to bring together data from previous molecular phylogenetic studies and combine them with newly produced sequences to: (i) reassess the circumscription of the cantharelloid clade; (ii) identify monophyletic groups within that clade; and (iii) determine whether the accelerated rate of molecular evolution in the rDNA of *Cantharellus, Craterellus* and *Tulasnella* also occurs in RPB2 and how rate variation affects inference of phylogenetic relationships in the clade. We hypothesized that the long-branch problem associated with the placement of *Cantharellus, Craterellus* and *Tulasnella* in earlier published rDNA phylogenies can be solved with the use of strict sequence alignments.

MATERIAL AND METHODS

We used 321 sequences of which 151 were from GenBank, 33 were from the AFTOL database, and 137 were new to this study (SUPPLEMENTARY TABLE I). Sequence data for each gene first were analyzed separately. We then conducted four analyses that optimized the sequence information available within subgroups: (i) *Cantharellus* only, all genes combined (19 strains);

(ii) *Sistotrema sensu lato*, nLSU data only (60 taxa); (iii) *Botryobasidium*-Ceratobasidiaceae, nLSU only (22 taxa); and (iv) *Tulasnella*, nLSU only (15 taxa). A combined all-taxa (except *Tulasnella*) four-gene dataset also was analyzed; it was composed of 34 taxa of which 26 had no missing data. Phylogenetic analyses employed both Bayesian Markov chain Monte Carlo and maximum parsimony bootstrapping methods. Combinability of the different data partitions was estimated explicitly from the incongruence length difference (ILD) test (Farris et al 1994) and empirically as described in Hofstetter et al (2002) and Miadlikowska and Lutzoni (2004). (See SUPPLEMENTARY INFORMATION.)

Three problems restrained us in constructing a ''supermatrix'' for a phylogenetic reassessment of the cantharelloid clade. First, many isolates had missing data at one or more loci. Second, we encountered several difficulties in the alignment of both nLSU and nSSU sequences from members of *Cantharellus, Craterellus* and *Tulasnella* with those from members of the other genera. Third, we found significant incongruence in the phylogenetic placement of *Tulasnella* depending on the gene analyzed (see SUPPLEMENTARY FIG. 1 and below). We attempted to reconstruct a ''supertree'' to bring together the separate (but optimized) analyses into a single phylogenetic tree, as described in Sanderson et al (1998). Only strongly supported nodes (0.95 pp or greater) were scored to create the matrix representation submitted to maximum parsimony analysis for a supertree reconstruction.

RESULTS AND DISCUSSION

The main objectives of this study were to reassess the circumscription of the cantharelloid clade (Hibbett and Thorn 2001, Binder et al 2005) and to identify monophyletic lineages within that clade. We produced many novel nLSU, nSSU, mtSSU and RPB2 sequences and combined them with data available in the NCBI and AFTOL public databases to conduct multiple phylogenetic analyses from both separate and concatenated datasets. (Results are presented in supplement.) They were generally consistent with earlier findings that used more limited taxa and character samplings and provided many novel insights about phylogenetic relationships within the clade.

Novel findings include the resolution of a core cantharelloid clade composed of at least three distinct lineages (FIG. 1): (i) *Cantharellus, Craterellus, Hydnum* and the *S. confluens-muscicola* group; (ii) *Clavulina, Membranomyces* and the *S. brinkmannii-oblongisporum* group; and (iii) the *S. eximum-octosporum* group. *Multiclavula* and other *Sistotrema* species also belong to that clade but their position was not fully resolved. We also demonstrate that *Sistotrema* is highly polyphyletic, that *Botryobasidium* is the sister group of the core cantharelloid clade and that Sebacinales do not belong to this clade. The latter finding solves the conflicting placement of this order between the studies of Weiß et al (2004a, b) and Binder et al (2005).

The phylogenetic position of *Tulasnella* was ambiguous. Data from nuclear rDNA genes placed this genus close to *Cantharellus* and *Craterellus*, whereas data from mtSSU and RPB2 placed it basal to the other cantharelloid taxa (SUPPLEMENTARY FIG. 1). The placement of *Tulasnella* indicated from mtSSU and RPB2 sequences is consistent with morphological evidence, in sharp contrast to its placement from nuclear rDNA data. We attribute the incongruent placement of *Tulasnella* by rDNA sequences to a long-branch attraction problem that results from an accelerated rate of molecular evolution in the nuclear RNA genes in *Cantharellus, Craterellus* and *Tulasnella*. Contrary to our expectation this problem still was present when only highly conserved gene regions were used in phylogenetic analyses, which necessitated the removal of respectively 53% and 35% of the aligned positions in the nLSU and nSSU data matrices (introns excluded, SUPPLEMENTARY TABLE II).

The removal of so many characters, which otherwise aligned well within subgroups, resulted in a significant loss of phylogenetic resolution within terminal clades. To elude this problem and also to avoid pitfalls associated with ''supermatrices'' containing many missing data (see Wiens 1998, 2003) we conducted multiple separate analyses and examined the possibility of using a ''supertree'' method (Sanderson et al 1998) to eventually combine these disparate datasets. Our attempt to reconstruct a meaningful supertree from the topologies (FIG. 1 and SUPPLEMENTARY FIG. 1) was largely unsuccessful (data not shown). This could be explained by the findings from a simulation study by Bininda-Emonds and Sanderson (2001) showing that ''the most important factor affecting supertree performance is, ironically, the most attractive feature of the method: the ability to combine trees with nonidentical taxon sets.'' We therefore agree with Gatesy et al (2004) who indicated that to address unsolved classification questions systematists should collect new character data rather than to make a supertree with limited data from the taxa of interest.

The core cantharelloid clade. Cantharellus *and* Craterellus.—The distinction between the genera *Cantharellus* and *Craterellus* (which collectively include about 90 described species) has long been disputed (Petersen 1971). Different authors classified some species in one genus or the other depending on which morphological characters were emphasized. Dahlman et al (2000) showed that these two genera can be

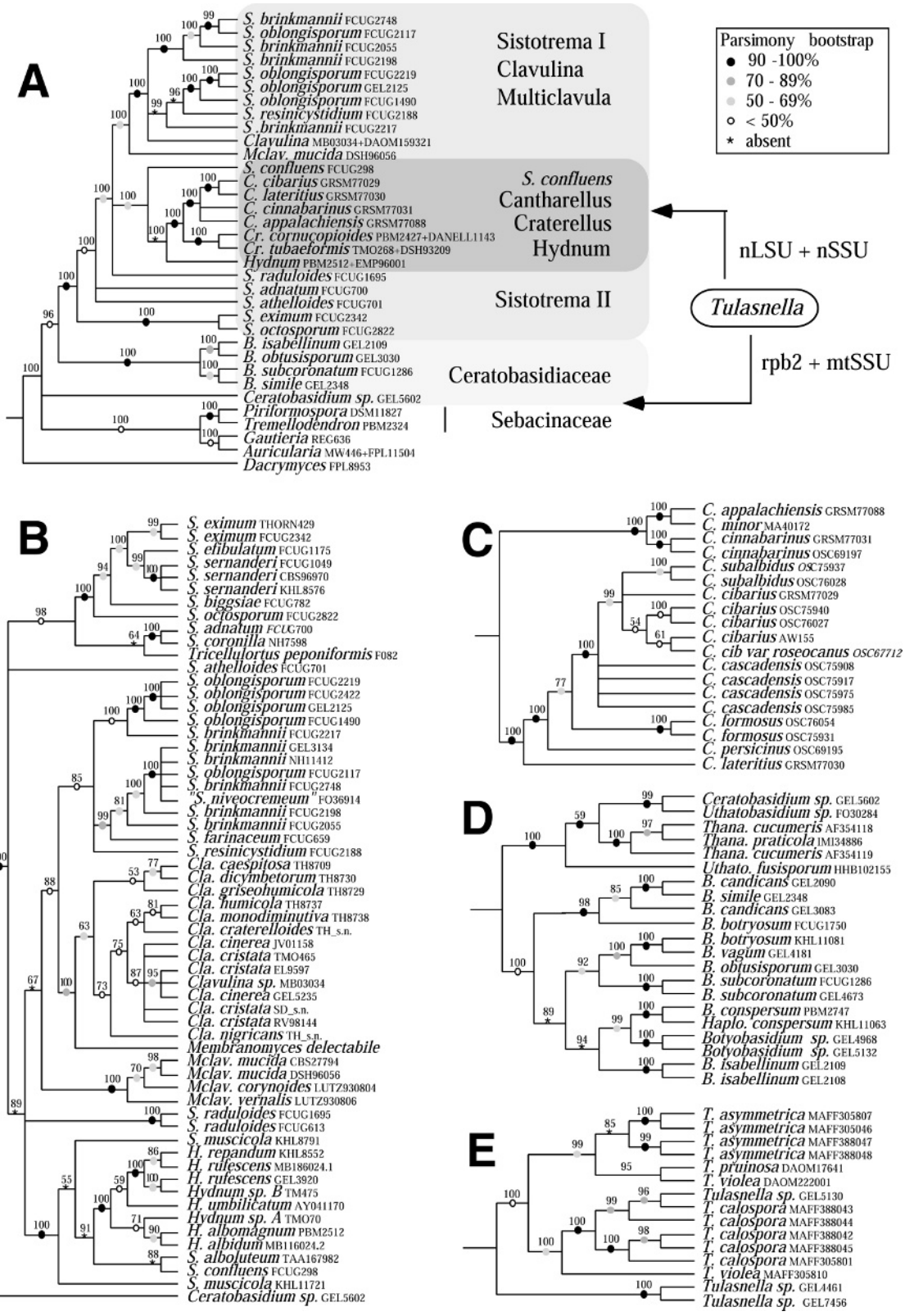

FIG. 1. Inferred phylogenetic relationships for: A. the all-taxa (minus *Tulasnella*) four-gene analyses showing the ambiguous placement of *Tulasnella* depending on which genes are analyzed; B. nLSU analyses in *Sistotrema* and close allies (minus *Cantharellus* and *Craterellus*); C. four-genes analyses in *Cantharellus* and *Craterellus*; D. nLSU analyses in *Botryobasidium* and Ceratobasidiaceae; E. nLSU analyses in *Tulasnella*. The trees are 50% majority rule Bayesian consensus. Bayesian posterior probabilities are shown above branches, and maximum-parsimony bootstrap supports are indicated by circles on branches.

distinguished based on nLSU and ITS sequences and that the presence of a hollow stipe seems to be a morphological synapomorphy for *Craterellus*. Results from the present study are in agreement with Dahlman et al (2000) and support a sister-group relationship between these two genera. Within *Craterellus* five species or species complexes can be recognized among northern temperate taxa: the *Cr. cornucopioides* complex (including *Cr. fallax* and *Cr. konradii*), the *Cr. tubaeformis* complex (including *Cr. infundibuliformis*), *Cr. odoratus*, *Cr. lutescens*, and *Cr. ignicolor* (Dahlman et al 2000).

A molecular phylogenetic study in *Cantharellus* was produced by Dunham et al (2003) from the use of nLSU and ITS data. Our four-gene phylogeny was in full agreement with the findings of these authors and supported the distinction between *C. cascadensis, C. formosus, C. subalbidus, C. persicinus, C. lateritius* and *C. cibarius*. Our tree (FIG. 1C) and other evidence (Moncalvo and Dunham unpublished) suggest the presence of several cryptic geographic species within the *C. cibarius* complex *sensu stricto*. A novel finding of this study is that two smaller, slender ''yellow chanterelles'', *C. appalachiensis* and *C. minor*, are more closely related to the red species of the *C. cinnabarinus* group than they are to the core group of yellow chanterelles.

Hydnum *and the* Sistotrema confluens *group.*—*Hydnum* is a morphologically well defined genus that includes about 120 described species characterized by fleshy fruiting bodies with a toothed or spinose hymenophore and pale, smooth spores. This genus, represented in our dataset by 11 strains representing at least seven species, was monophyletic in all our analyses. Two closely related species commonly reported throughout the northern hemisphere, *H. repandum* and *H. rufescens*, were not found to be respectively monophyletic and warrant further comparative taxonomic scrutiny from a global geographic sampling (FIG. 1B).

Strongly clustering with *Hydnum* in the nLSU analyses were *Sistotrema confluens*, *S. alboluteum* and *S. muscicola* (FIG. 1B). These species (along with *S. dennisii*, not sampled here) are distinguished from other *Sistotrema* species by the presence of a irpicoid-poroid hymenophore (sometime almost hydnoid in the case of *S. confluens*), globose to subglobose spores and lack of cystidia (Eriksson et al 1984). The placement of *S. confluens* and *S. muscicola* close to *Hydnum* was indicated already in Larsson et al (2004). Our two samples of *S. muscicola* were quite divergent (SUPPLEMENTARY FIG. 1). According to Eriksson et al (1984) much controversy still surrounds the true identity and circumscription of *S. muscicola*, which should only be regarded as a ''form-complex''. Based on their phylogenetic affinities, we suspect these *Sistotrema* species to be ectomycorrhizal.

Phylogenetic relationships among the chanterelles, *Hydnum* and the *S. confluens* group remained unclear. In the all-taxa four-gene analyses (SUPPLEMENTARY FIG. 1), the Bayesian tree strongly supported *Hydnum* as the sister group of *Cantharellus-Craterellus* (Bayesian posterior probability [pp] = 1) while parsimony bootstrapping suggested *S. confluens* as the sister group (88% bootstrap support [bs]).

Sistotrema traditionally has been regarded as a relatively well delimited genus of wood saprophytes characterized by the presence of urniform basidia generally bearing 6–8 sterigmata, but species limits are often unclear (Eriksson et al 1984). Most *Sistotrema* species have a corticioid habit with a smooth or somewhat irregularly poroid or irpicoid-hydnoid hymenophore, but some species develop sporocarps that mimic the dimidiate or stipitate habits. Results from our analyses clearly demonstrated that *Sistotrema* is highly polyphyletic (FIG. 1A–B). Nonmonophyly of this genus already was suggested in the studies of Larsson et al (2004) and Binder et al (2005).

Sistotrema is *polyphyletic.*—Phylogenetic relationships of *Sistotrema* species with an irpicioid-poroid hymenium (*S. confluens*, *S. alboluteum, S. muscicola*) to *Hydnum* was discussed above. Because *S. confluens* is the type species of the genus, the species presented below are in need of nomenclatural revision. Our analyses revealed three monophyletic groups (the *S. brinkmannii-oblongisporum* clade, the *S. eximum-octosporum* clade and *S. adnatum-coronilla*) and left two species with unresolved phylogenetic affinities (*S. raduloides* and *S. athelioides*).

S. brinkmannii, S. farinaceum, S. resinicystidium and *S. oblongisporum* form a monophyletic group (FIG. 1B), but there is no obvious morphological synapomorphy to arrange these taxa together. The morphological species *S. brinkmannii* was found to consist of an aggregate of biological species (Lemke 1969, Ullrich and Raper 1975, Hallenberg 1984). This is concordant with our tree (FIG. 1B) that shows nonmonophyly of isolates that were identified morphologically as *S. brinkmannii,* which mixed with strains labeled *S. oblongisporum*. The sequence labeled *Sistotremastrum niveocremeum* that nested in this group represents a misidentification; the true *Sistotremastrum niveocremeum* belongs to the trechisporoid clade (Binder et al 2005, Larsson unpublished).

Another monophyletic group consisted of *S. eximum, S. efibulatum, S. sernanderi, S. biggsiae* and *S. octosporum* (FIG. 1B). No obvious morphological

evidence groups these taxa together (Eriksson et al 1984). Our analyses also indicated monophyly of strains labeled *S. adnatum* and *S. coronilla*, which clustered with the *S. eximum* group in the nLSU analyses (FIG. 1B) but not in the all-taxa four-gene analyses (SUPPLEMENTARY FIG. 1). *S. coronilla* was noted as a doubtful species by Eriksson et al (1984) and sometimes was listed as a synonym of *S. brinkmannii*. Weakly clustering with *S. adnatum* and *S. coronilla* was a sequence labeled *Tricellulortus peponiformis* (AY004068, Platas et al unpublished; correct genus name is *Pneumatospora*). This species represents a monotypic anamorphic genus classified in the Basidiomycota in the Index of Fungi (http://www.indexfungorum.org) but listed as a mitosporic ascomycete in the NCBI taxonomic database (http://www.ncbi.nlm.nih.gov). Further investigation on the identity and phylogenetic relationships of this poorly known taxon is needed.

Our analyses placed *Sistotrema raduloides* and *S. athelioides* in more basal, unresolved position in the cantharelloid clade *sensu stricto* (FIG. 1A–B). *S. raduloides* is a circumboreal species forming extended, distinctly hydnoid sporocarps, preferably on dead aspen logs. *S. athelioides* is known only from one locality on Vancouver Island, British Columbia, and was described as one of many genetically distinct forms within the *S. brinkmannii* complex (Hallenberg 1984). The fact that these two species clustered separately from the other *Sistotrema* species further demonstrated the high heterogeneity of the genus.

Overall our results demonstrate the need for a more detailed study of the urniform-bearing basidia genus ''*Sistotrema*'', which appears to be a polyphyletic assemblage of essentially resupinate forms from which coralloid, hydnoid and agaricoid sporocarps have evolved.

Clavulina *and* Membranomyces.—The coralloid genus *Clavulina* is characterized by branched basidiomata and contains at least 50 species worldwide, primarily in the tropics (Henkel et al 2005). It traditionally was segregated from other coral fungi by the presence of cornute, bisterigmate basidia (Corner 1950, Petersen 1988). However neotropical species with unbranching basidiomata and/or forming infundibuliform rather than coralloid basidiomes and/or bearing 4–6 spores per basidium recently were described and their classification in *Clavulina* was supported by nLSU sequence data (Thacker and Henkel 2004, Henkel et al 2005). The placement of *Clavulina* in the cantharelloid clade first was indicated by Hibbett et al (1997) and substantiated in several subsequent studies. Here we sampled more broadly within this genus and confirm the monophyly of *Clavulina sensu* Henkel and collaborators and indicate that this genus is sister of the *S. brinkmannii-oblongisporum* clade (FIG. 1A–B).

The small corticioid genus *Membranomyces* (two spp.) exhibits cylindrical basidia with cornute sterigmata, and subglobose, smooth, slightly thick-walled spores as *Clavulina*. Based on these similarities Parmasto proposed the transfer of this corticiaceous genus to the Clavulinaceae (Eriksson and Ryvarden 1973). Our results indicate a sister relationship between *Clavulina* and *Membranomyces* (FIG. 1B) as in Larsson et al (2004) and Binder et al (2005). However this assumption still is based solely on a single nLSU sequence of *Membranomyces delectabilis* (AY586688, Larsson et al 2004). This species originally was referred to the genus *Clavulicium* that is typified by *Clavulicium macounii*. Jülich (1975) questioned this generic arrangement and created *Membranomyces* to segregate simple-septate species. Molecular data support that decision because *Clavulicium* does not belong to the cantharelloid clade although its phylogenetic position still is unresolved (K-H Larsson unpublished).

Multiclavula.—The small, lichenized club-mushroom genus *Multiclavula* currently consists of 12 accepted species (Index of Fungi). This genus has been found affiliated with cantharelloid taxa in many previous molecular phylogenetic studies, but its position within the clade has remained unclear. Here we present the first evidence that *Multiclavula* is the sister group of *Clavulina* and the *S. brinkmannii-oblongisporum* clade (FIG. 1A–B).

Botryobasidium.—Species of the saprophytic genus *Botryobasidium* have corticioid to hypochnoid resupinate basidiocarps and characteristic basidia that are short, cylindrical or subcylindrical to suburniform with 2–8 sterigmata, and generally arranged in clusters (Eriksson and Ryvarden 1973). Anamorphic stages are known and were described in *Haplotrichum* or *Allescheriella*. *Botryobasidium* was monographed by Langer (1994), who accepted 48 species in the genus. Parmasto et al (2004) similarly recognized 50 species. Relationships among *Botryobasidium*, *Sistotrema*, the Ceratobasidiaceae and *Tulasnella* have long been suggested and debated (Martin 1948; Donk 1956, 1972; Parmasto 1968; Eriksson and Ryvarden 1973; Jülich 1981). These taxa share similar short or urniform basidia that also often deviate from the 4-sterigmata type that is common to most homobasidiomycetes.

The first molecular evidence of a close phylogenetic relationship between *Botryobasidium* and *Cantharellus* was presented by Hibbett et al (1997). Here we sampled sequence data from 17 members of *Botryobasidium* representing at least 10 species (SUPPLEMEN-

TARY TABLE I and FIG. 1D). Monophyly of this genus was supported strongly (100% bs/pp = 1), in agreement with Binder et al (2005) who sampled 11 isolates from this genus. Our four-gene analyses suggested that *Botryobasidium* is a sister group of the core cantharelloid clade (FIG. 1A). It also appears that the taxonomic identity of, and distinction among, *B. candicans*, *B. botryosum* and *B. simile* are problematic (FIG. 1D), as pointed by Eriksson and Ryvarden (1973).

The Ceratobasidiaceae.—A major problem in the phylogeny of Hymenomycetes concerns the placements of the Ceratobasidiaceae (= Ceratobasidiales *sensu* Roberts 1999), Tulasnellales and Sebacinales (Hibbett 2003). The Ceratobasidiaceae includes the genera *Ceratobasidium*, *Thanatephorus, Uthatobasidium, Waitea* and *Marchandiobasidium*, which presently are composed of respectively 21, nine, two, two and one recognized species (Index of Fungi). These corticioid taxa are united by the presence of a perforate parenthesome with large openings. Also, except in the small genera *Waitea* and *Marchandiobasidium* and in a few *Thanatephorus* species, these taxa form secondary spores (or ''spore germinating by repetition'') from primary spores born on short, often urniform holobasidia (Roberts 1999, Diederich et al 2003, Weiß et al 2004a). The formation of secondary spores is a well known phenomenon in the heterobasidiomycetes but is not observed in typical homobasidiomycetes. This particular feature led Donk (1964, 1972) and others (e.g. Eriksson and Ryvarden 1973) to link *Ceratobasidium* to the heterobasidiomycetes, particularly to *Tulasnella*. However the 2–8-sterigmate and urniform basidia along with the corticioid habit deterred these authors from decisively separating these taxa from *Sistotrema*, *Botryobasidium* and the Corticiaceae *sensu lato*.

Marchandiobasidium is a sclerotium-producing lichenicolous fungus that recently was segregated from the form-genus *Marchandiomyces* and classified in the Ceratobasidiales by Diederich et al (2003), in part because they noted that the unidentified nSSU sequence clustering with a *Thanatephorus* sequence in Sikaroodi et al (2001) corresponds to *Marchandiobasidium aurantiacus*. The SSU phylogeny presented in Sikaroodi et al (2001) also placed the anamorph of the type of *Waitea*, *Rhizoctonia zeae*, in an unresolved position but well separated from *Thanatephorus*. *Waitea* was placed with *Piloderma* at the base of the Agaricales in Bruns et al (1998). These results suggest that *Waitea* does not belong to the Ceratobasidiaceae.

Overall it appears that the Ceratobasidiales *sensu* Roberts (1999) is probably polyphyletic. The core taxa of the traditional Ceratobasidiaceae (*Ceratobasidium*, *Thanatephorus* and *Uthatobasidium*) however seem to represent a monophyletic group that belongs to the cantharelloid clade (see below). But the taxonomic situation is complicated by the fact that the type species of *Ceratobasidium* (*C. calosporum*) has a dolipore with an imperforate parenthesome, whereas the ultrastructural circumscription of the Ceratobasidiales by Roberts (1999) was based on the presence of perforated parenthesomes with large openings (Weiß et al 2004a). A major problem in dealing with the systematics of these fungi is that accurate taxonomic identification is difficult using morphology alone. In addition DNA sequence sampling for members of this group still is limited to a few isolates.

Here we used Ceratobasidiaceae sequences available from public databases and found that they form a monophyletic group that is sister of both *Botryobasidium* and members of the core cantharelloid clade (FIG. 1A). Our results also showed that the distinction between *Uthatobasidium* and *Ceratobasidium* is not clear-cut (FIG. 1D) and will need further investigation. Also *Thanatephorus* mainly was distinguished from *Uthatobasidium* for being parasitic on herbaceous plants and its connection to *Rhizoctonia* anamorphs (Hjortstam et al 1988), but a recent molecular phylogenetic study by Gonzalez et al (2001) showed that *Rhizoctonia* anamorphs are associated with both *Ceratobasidium* and *Thanatephorus* teleomorphs. In summary much more work is required to resolve evolutionary relationships and taxonomic concepts within the Ceratobasidiaceae/Ceratobasidiales.

Tulasnella.—The traditional heterobasidiomycete genus *Tulasnella* and related taxa (Tulasnellaceae or Tulasnellales) consist of resupinate forms characterized by unique basidia with swollen septate epibasidia in place of sterigmata, which produce secondary spores by the process of germinating by repetition. The genus currently includes 47 described species (Index of Fungi) and many *Rhizoctonia* anamorphs (Roberts 1999). *Tulasnella* forms plant ectomycorrhizae and mycorrhiza-like associations with liverworts (Bidartondo et al 2003, Kottke et al 2003) and also is associated with orchid roots along with other *Rhizoctonia*-forming fungi with teleomorphs in the Ceratobasidiaceae and Sebacinales (Rasmussen 1995, Roberts 1999, Kristiansen et al 2001, Taylor et al 2003, Bidartondo et al 2004, Shefferson et al 2005).

Tulasnella first was proposed to be a member of the cantharelloid clade in Hibbett and Thorn (2001). This placement was confirmed in later studies that used nuclear rDNA sequences (e.g. Bidartondo et al 2003, Kottke et al 2003, Weiß et al 2004, Binder et al

2005), but its exact position within that clade remained unclear. Problems associated with high rate of molecular evolution in *Tulasnella* nuclear rDNA genes have been discussed above (and in SUPPLEMENT). Here we showed that mtSSU, and more robustly RPB2, sequence data placed *Tulasnella* as a sister group of all the taxa presented above. This inferred phylogenetic position, along with both morphological (resupinate habit and spore germination with repetition) and ecological (*Rhizoctonia*-type orchid association) evidences, collectively support the placement of *Tulasnella* in a more basal position in the cantharelloid clade, in the vicinity of Ceratobasidiaceae. Such placement also agrees with the nonmonophyly of the heterobasidiomycetes as it appeared from recent studies (Weiß and Oberwinkler 2001; Weiß et al 2004a, b; Lutzoni et al 2004; Matheny and Hibbett unpublished). It also reconciles the dilemma of past authors about the relationships among Ceratobasidiaceae, *Botryobasidium* and *Tulasnella,* as discussed in Eriksson and Ryvarden (1973:219).

Sebacinales.—Sebacinales (Weiß et al 2004b) are traditional heterobasidiomycetes with longitudinally septate exidioid basidia (Wells and Oberwinkler 1982). Members of this order are involved in a wide spectrum of mycorrhizal associations with plants and liverworts (Rasmussen 1995; Roberts 1999; Kristiansen et al 2001; Taylor et al 2003; Bidartondo et al 2003, 2004; Kottke et al 2003; Taylor et al 2003; Weiß et al 2004b; Setaro et al 2006). Our analyses support monophyly of the Sebacinales (represented here with the genera *Sebacina, Serendipita, Craterocolla, Piriformospora* and *Tremellodendron*) as in Weiß and Oberwinkler (2001) and Weiß et al (2004a, b). While the present study supports the inclusion of the Ceratobasidiaceae and possibly also *Tulasnella* in the cantharelloid clade, our results show no evidence to place the Sebacinales in that clade. In the all-taxa four-gene analyses, our representatives of the Sebacinales (*Piriformospora indica* and *Tremellodendron pallidum*) strongly clustered with *Gautieria* (representing the gomphoid-phalloid clade) and *Auricularia* (a traditional heterobasidiomycete) when the tree is rooted with *Dacrymyces* (heterobasidiomycetes). The latter relationships should be taken with much caution because in this study our sampling of gomphoid-palloid and heterobasidiomycetes was limited.

CONCLUSION

The cantharelloid clade represents an ancient hymenomycete lineage composed of morphologically and ecologically diverse fungi (FIG. 2). A possible synapomorphy for this clade could be the stichic type of nuclear division (Hibbett and Thorn 2001, Larsson et al 2004) that was found in *Cantharellus, Craterellus, Clavulina, Membranomyces* and *Hydnum* (Penancier 1961). However information about the nuclear division type in *Sistrotrema, Botryobasidium* and Ceratobasidiaceae still is lacking. *Tulasnella* species display chiastic nuclear division (Penancier 1961). This cytological character reinforces the mtSSU and RPB2 phylogenies displacing *Tulasnella* from *Cantharellus-Craterellus* and the core cantharelloid group, in conflict with rDNA data (SUPPLEMENTARY FIG. 1). Parenthesome ultrastructure has been considered a possible character for recognizing major basidiomycete lineages (Clémençon 1997). Perforate parenthesomes are found commonly in the homobasidiomycetes, while imperforate parenthesomes characterize the traditional heterobasidiomycetes (e.g. Dacrymycetales, Auriculariales, Sebacinales and Tulasnellales). Imperforate parenthesomes however also occur in several members of the gomphoid-phalloid, hymenochaetoid, trechisporoid and cantharelloid clade. In the cantharelloid clade imperforate parenthesomes have been found in *Cantharellus* and *Botryobasidium,* but perforate parenthesomes have been reported from Ceratobasidiales (except for the type species of *Ceratobasidium,* see above) and *Sistotrema brinkmannii* (Langer 1994, Hibbett and Thorn 2001, Weiß and Oberwinkler 2001, Diederich et al 2003, Larsson et al 2004, Bianchinotti et al 2005). Therefore no single parenthesome type unites members of the cantharelloid clade.

This was the first study using sequence data from a protein-coding gene (RPB2) for molecular systematics in the cantharelloid clade. Results indicate that, compared to rDNA genes, RPB2 provides a higher proportion of variable and parsimony informative characters (SUPPLEMENTARY TABLE I), has a more uniform among-taxa rate of evolution (SUPPLEMENTARY FIG. 1) and better resolves phylogenetic relationships within the clade (data not shown). We therefore recommend the use of this and other protein-coding genes in future molecular phylogenetic studies of the cantharelloid clade. This clade is ancient and morphologically and ecologically diverse. A robust phylogeny for this group of fungi therefore will be highly valuable for inferring the state of ancestral characters in the hymenomycetes and their evolution. For instance a fully resolved phylogeny of the cantharelloid clade could shed new light on the origin of the holobasidia and on the much debated questions whether the first hymenomycetes were free-living or symbiotic.

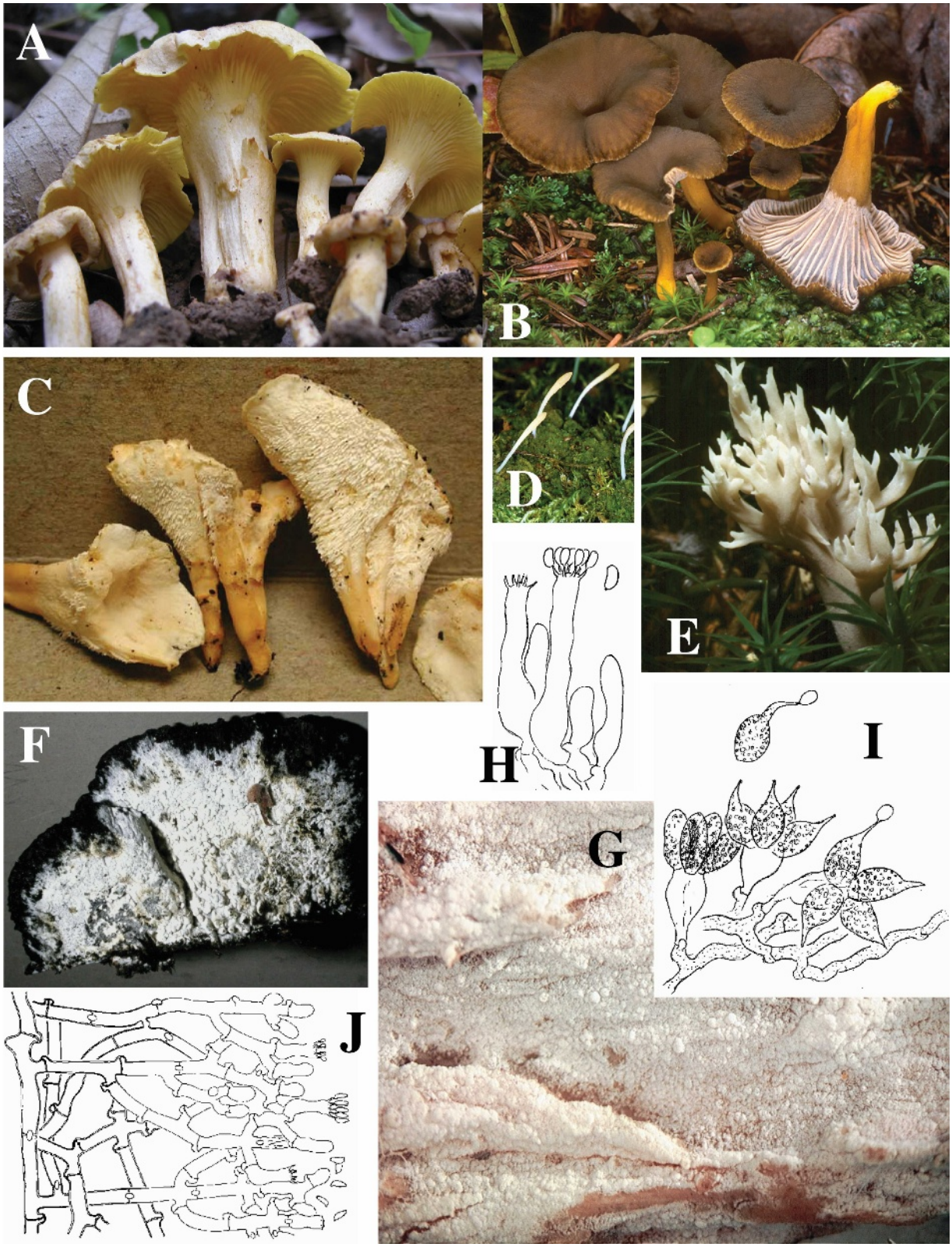

FIG. 2. Morphological diversity in the cantharelloid clade. Basidiocarps of: A. *Cantharellus* aff. *cibarius* (image from J.-M. Moncalvo); B. *Craterellus tubaeformis* (M. Wood); C. *Sistotrema confluens* (R. Halling); D. *Multiclavula mucida* (M. Wood); E. *Clavulina cinerea* (E. Langer); F. *Botryobasidium subcoronatum*, fruiting on an old polypore (E. Langer); G. *Sistotrema coroniferum* (K.-H. Larsson). Basidia and spores of: H. *Sistotrema brinkmannii* (E. Langer); I. *Tulasnella inclusa* (E. Langer); J. *Botryobasidium subcoronatum* (E. Langer).

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SUPPLEMENTARY INFORMATION

MATERIAL AND METHODS

Source of data.—We first retrieved from *mor* (http://mor.clarku.edu; Hibbett et al 2005) nLSU sequences belonging to the cantharelloid clade, then searched both the NCBI and AFTOL nucleotide databases for additional nLSU as well as nSSU, mtSSU and RPB2 sequences. Many of the retrieved sequences were used as query sequences in BLAST searches in both the NCBI and AFTOL databases to (i) confirm taxonomic and sequence accuracy and (ii) retrieve additional sequences putatively belonging to the cantharelloid clade. Using this initial dataset, we identified target taxa and genes useful to broadening both the taxonomic and genomic coverage in the clade. Novel sequence data were produced in different laboratories using various standard protocols for DNA extraction, PCR amplification, DNA sequencing and sequence editing. The strains and sequences used in the final analyses are provided (SUPPLEMENTARY TABLE I). In total sequences from the nLSU, nSSU, mtSSU and RPB2 genes were available respectively for 140, 75, 62 and 46 taxa. Of these 323 sequences, 151 were from GenBank, 33 were obtained directly from the AFTOL database and 137 are new to this study. Novel sequences however were largely biased toward *Sistotrema* (69 sequences) and *Cantharellus* (36), and many isolates lacked sequences from both the mtSSU and RPB2 loci. Analyses included representative members of the gomphoid-phalloid clade (*Gomphus*, *Ramaria* and *Gautieria*) and trees were rooted with sequences from *Dacrymyces* and *Auricularia*.

Phylogenetic analyses.—Sequences were aligned in Clustal W (Thompson et al 1994) followed by manual optimization in SEAL v2.0a11 (Rambaut 1996). All ambiguously aligned regions were removed before phylogenetic analyses. Both Bayesian Markov chain Monte Carlo (B-MCMC) and maximum parsimony bootstrapping (MPB) analyses were conducted on all datasets. Bayesian analyses were performed in MrBayes 3.1.1 (Ronquist and Huelsenbeck 2003, Altekar et al 2004) with a 28-node Linux Beowulf cluster, using a general time-reversible model of DNA substitution with these settings: six classes of nucleotide substitutions, gamma rate among sites, four Monte Carlo Markov chains run for 1 000 000 generations starting from random trees and sampling one tree every 100 generations. The first 1000 sampled trees were discarded (burn-in). The resulting 50% majority rule tree was computed and viewed in PAUP* 4.0b10 (Swofford 2003). MPB analyses were conducted in PAUP* with the use of heuristic search methods and these settings: 1000 bootstrap replicates of one random addition sequence each, no more than 10 trees kept per replicate, TBR branch-swapping and retention of groups compatible with 50% majority-rule consensus. Combinability of the different data partitions was estimated with the incongruence length difference (ILD) test (Farris et al 1994) as implemented under the name of partition-homogeneity test in PAUP*. Heuristic search settings for the ILD test were set to 100 replicates of one random addition sequence keeping no more than 10 trees per replicates and TBR branch-swapping. Because the ILD test has been widely criticized (Cunningham 1997, Barker and Lutzoni 2002, Darlu and Lecointre 2002), data combinability also was evaluated empirically by considering whether separate tree topologies conflicted in strongly supporting the monophyly of incompatible groups (Hofstetter et al 2002, Miadlikowska and Lutzoni 2004).

Analytical strategy and datasets analyzed.—Three problems restrained us in constructing a ''supermatrix'' for a phylogenetic reassessment of the cantharelloid clade. First, we encountered several difficulties in the alignment of both nLSU and nSSU sequences from members of *Cantharellus, Craterellus* and *Tulasnella* with those from members of the other genera. These difficulties necessitated the exclusion of many ambiguously aligned characters (53% and 35% in the nLSU and nSSU matrices respectively). However many characters aligned well within subgroups and were useful for inferring evolutionary relationships in separate analyses of subgroups. Second, many isolates had missing data at one or more loci. Wiens (1998, 2003) reported the many problems associated with the use of ''supermatrices'' that include many missing data, one of them being significant decrease in statistical confidence for nodes in tree topologies. Third, we found significant incongruence in the phylogenetic placement of *Tulasnella* depending on the dataset analyzed (see below). We therefore conducted both separate and combined gene analyses (SUPPLEMENTARY TABLE II). When combining the different gene datasets we emphasized character rather than taxon sampling.

Our combined all-taxa four-gene dataset was composed of 34 taxa (after exclusion of *Tulasnella*, see below) as follows: 26 strains had no missing data; four had missing data for one gene (*Ceratobasidium* sp. and *Multiclavula mucida* DSH96056 lacked RPB2 sequences, and *Tremellodendron pallidum* and *Dacrymyces* sp. lacked mtSSU sequences); five strains had missing mtSSU data and were complemented with a mtSSU sequence from a different conspecific or congeneric strain after verification that their respective sequences for the other genes were strongly clustering together in separate gene analyses. These were *Clavulina* sp. MB03034 (mtSSU sequence from *C. cristata* DAOM159321), *Hydnum albomagnum* PBM2512 (mtSSU sequence from *H. repandum* EMP96001), *Craterellus cornucopioides* PBM2427 (mtSSU from the conspecific isolate DANELL1143), *Craterellus tubaeformis* TMO268 (mtSSU from the conspecific isolate DSH93209), and *Auricularia auricula-judae* MW446 (mtSSU from the conspecific isolate FPL11504).

The data matrices used in subclade analyses retained significantly more characters than the all-taxa matrices (SUPPLEMENTARY TABLE II). Based on the sequences available, these four subclade analyses were conducted: *Cantha-*

SUPPLEMENTARY TABLE I. Sequence data used in this study, their origin and their GenBank accession number

TAXA	STRAIN	SOURCE OF DATA [a]	nLSU	nSSU	mtSSU	RPB2
CANTHARELLUS						
appalachiensis	GRSM 77088	1	DQ898690	DQ898668	DQ898646	DQ898748
cascadensis	OSC 75985	2, 1	AY041162	DQ898688	DQ898678	n/a
cascadensis	OSC 75975	2, 1	AY041163	DQ898689	DQ898677	n/a
cascadensis	OSC 75917	2, 1	AY041158	n/a	DQ898675	n/a
cascadensis	OSC 75908	2, 1	AY041160	n/a	DQ898676	n/a
cibarius	OSC 75940	2, 1	AY041157	DQ898684	DQ898673	n/a
cibarius	OSC 76027	2, 1	AY041155	n/a	DQ898674	n/a
cibarius	GRSM 77029	1	DQ898693	DQ898670	DQ898647	DQ898745
cibarius	AW 155	3	AFTOL	n/a	n/a	AFTOL
cib. var. roseocanus	OSC 67712	2, 1	AY041152	DQ898685	DQ898679	n/a
cinnabarinus	GRSM 77031	1	DQ898692	DQ898669	DQ898649	DQ898747
cinnabarinus	OSC 69197	2	AY041168	n/a	n/a	n/a
formosus	OSC 76054	2, 1	AY041165	DQ898686	DQ898681	n/a
formosus	OSC 75931	2	AY041166	n/a	n/a	n/a
lateritius	GRSM 77030	1	DQ898694	DQ898671	DQ898648	DQ898746
minor	MA 40172	1	DQ898691	DQ898672	DQ898650	n/a
persicinus	OSC 69195	2	AY041169	n/a	n/a	n/a
subalbidus	OSC 75937	2, 1	AY041149	DQ898687	DQ898680	n/a
subalbidus	OSC 76028	2	AY041150	n/a	n/a	n/a
CRATERELLUS						
aurora	UPSF 11791	4	AF105304	n/a	n/a	n/a
cornucopioides (fallax)	PBM 2427	3	AY700188	AY771604	n/a	AFTOL
cornucopioides	Danell 1143	5	n/a	AF184190	AF185976	n/a
cornucopioides	UPSF 11800	4	AF105298	n/a	n/a	n/a
cornucopioides	DSH 96-003	5	n/a	AF184191	AF185977	n/a
ignicolor	UPSF-11794	4	AF105314	n/a	n/a	n/a
lutescens	DAOM 199243	5	n/a	AF184177	AF185976	n/a
tubaeformis	TM 0268	1	DQ898741	n/a	DQ898651	DQ898749
tubaeformis	DSH 93-209	6, 7	AF287851	AF026636	AF026678	n/a
tubaeformis	OSC 49915	1	n/a	DQ898683	DQ898682	n/a
SISTOTREMA						
brinkmannii	FCUG 2055	1	DQ898706	DQ898712	DQ898654	DQ898754
brinkmannii	FCUG 2748	1	DQ898704	DQ898714	DQ898652	DQ898752
brinkmannii	FCUG 2198	1	DQ898705	DQ898713	DQ898653	DQ898753
brinkmannii	FCUG 2217	1	DQ898709	DQ898715	DQ898655	DQ898755
brinkmannii	GEL 3134	8	AJ406430	n/a	n/a	n/a
brinkmannii	NH 11412	9	AF506473	n/a	n/a	n/a
farinaceum	FCUG 659	1	DQ898707	DQ898718	n/a	DQ898756

SUPPLEMENTARY TABLE I. Continued

TAXA	STRAIN	SOURCE OF DATA [a]	nLSU	nSSU	mtSSU	RPB2
oblongisporum	FCUG 2117	1	DQ898703	DQ898717	DQ898658	DQ898759
oblongisporum	FCUG 1490	1	DQ898702	DQ898716	DQ898657	DQ898758
oblongisporum	FCUG 2219	1	DQ898701	DQ898719	DQ898656	DQ898757
oblongisporum	FCUG 2422 = AFTOL 617	3	AFTOL	AY757263	n/a	n/a
oblongisporum	GEL 2125	1	DQ898728	DQ898738	DQ898732	DQ898767
resinicystidium	FCUG 2188	1	DQ898708	DQ898720	DQ898659	DQ898760
sp. (as ''niveocremeum'' in GenBank)	FO 36914	8	AJ406429	n/a	n/a	n/a
eximum	THORN 429	10, 11	AF393076	n/a	n/a	AY218518
eximum	THORN 420	12	n/a	AF334935	AF334891	n/a
eximum	FCUG 2342 = AFTOL 616	1 (3)	DQ898695	AY757261	DQ898660	DQ898762
sernanderi	FCUG 1049	3	AFTOL	AY757264	n/a	n/a
sernanderi	KHL 8576	9	AF506476	n/a	n/a	n/a
sernanderi	CBS 969.70	12	AF518650	n/a	AF334893	n/a
efibulatum	FCUG 1175	1	DQ898696	DQ898721	DQ898661	n/a
biggsiae	FCUG 782	1	DQ898697	DQ898723	DQ898662	n/a
octosporum	FCUG 2822	1	DQ898698	DQ898722	DQ898663	DQ898764
athelioides	FCUG 701	1	DQ898700	DQ898724	DQ898664	DQ898766
adnatum	FCUG 700	1	DQ898699	DQ898725	DQ898665	DQ898763
coronilla	FCUG 863 = AFTOL 618	3	n/a	AY757259	n/a	AFTOL
coronilla	NH 7598	9	AF506475	n/a	n/a	n/a
muscicola	FPL 8233	13, 12, 11	AF518649	AF334936	AF334892	AY218519
muscicola	KHL 8791	14	AF506474	n/a	n/a	n/a
muscicola	KHL 11721	15	AJ606040	n/a	n/a	n/a
alboluteum	TAA 167982	14	AY586713	n/a	n/a	n/a
confluens	FCUG 298 = AFTOL 613	1	DQ898711	DQ898726	DQ898666	DQ898761
raduloides	FCUG 1695	1	DQ898710	DQ898727	DQ898667	DQ898765
raduloides	FCUG 613	3	AY647213	AY757262	n/a	n/a
PNEUMATOSPORA						
peponiformis (as TRICELLULORTUS)	F-082,316	16	AY004068	n/a	n/a	n/a
HYDNUM						
albidum	MB 11-6024/2	17	AY293186	AY293136	n/a	n/a
albomagnum	PBM 2512 = AFTOL 471	3	AY700199	AY665777	n/a	DQ234553
repandum	DSH 97-320	3, 11	n/a	AFTOL	n/a	AY218489
repandum	KHL 8552	14	AF347095	n/a	n/a	n/a

SUPPLEMENTARY TABLE I. Continued

TAXA	STRAIN	SOURCE OF DATA [a]	nLSU	nSSU	mtSSU	RPB2
repandum	EMP 96-001	7	n/a	AF026641	AF026683	n/a
repandum	MTS 3757	18	n/a	n/a	n/a	AY485624
rufescens	MB 18-6024/1	17	AY293187	AY293136	n/a	n/a
rufescens	GEL 3920	8	AJ406427	n/a	n/a	n/a
sp. A	TM 070	1	DQ898744	n/a	n/a	DQ898750
sp. B	TM 475	1	DQ898743	n/a	n/a	DQ898751
umbilicatum	n/a	2	AY041170	n/a	n/a	n/a
MEMBRANOMYCES						
delectabile	KHL 11147	14	AY586688	n/a	n/a	n/a
CLAVULINA						
caespitosa	TH 8709	19	DQ056370	n/a	n/a	n/a
cinerea	WTU JFA-10798	5	n/a	AF184186	AF185974	n/a
cinerea	GEL 5235	8	AJ406433	n/a	n/a	n/a
cinerea	JV 01-158	20	AJ889937	n/a	n/a	n/a
craterelloides	n/a	21	AY391718	n/a	n/a	n/a
cristata	TM 465	1	DQ898742	n/a	n/a	n/a
cristata	EL 95_97	14	AY586648	n/a	n/a	n/a
cristata	RV 98/144	22	AF261553	n/a	n/a	n/a
cristata	n/a	2	AY041171	n/a	n/a	n/a
cristata	DAOM 159321	7	n/a	AF026640	AF026682	n/a
dicymbetorum	TH 8730	19	DQ056369	n/a	n/a	n/a
griseohumicola	TH 8729	19	DQ056366	n/a	n/a	n/a
humicola	TH 8737	19	DQ056367	n/a	n/a	n/a
monodiminutiva	TH 8738	19	DQ056372	n/a	n/a	n/a
nigricans	n/a	21	AY391719	n/a	n/a	n/a
sp.	MB 03-034 = AFTOL 667	3	AY745694	AY757265	n/a	AFTOL
MULTICLAVULA						
corynoides	Lutzoni 930804-2	23	U66440	n/a	n/a	n/a
mucida	CBS 277.94 = AFTOL 1130	3	AY885163	n/a	n/a	n/a
mucida	DSH 96-056	6, 7	AF287875	AF026613	AF026659	n/a
mucida	n/a	24	n/a	U23542	n/a	n/a
vernalis	Lutzoni 930806-1	23	U66439	n/a	n/a	n/a
CERATOBASIDIACEAE						
Ceratobasidium sp.	GEL 5602 = AFTOL 608	3, 17	AY293171	AY757266	AY293223	n/a
Thanatephorus cucumeris	AG4-HGI AH-1	25	AF354118	n/a	n/a	n/a
Thanatephorus cucumeris	AG8 A68	25	AF354119	n/a	n/a	n/a

SUPPLEMENTARY TABLE I. Continued

TAXA	STRAIN	SOURCE OF DATA [a]	nLSU	nSSU	mtSSU	RPB2
Thanatephorus cucumeris	Maria FCC93	26	n/a	AY946268	n/a	n/a
Thanatephorus praticola	IMI 34886	13	AF518655	n/a	n/a	n/a
Uthatobasidium fusisporum	HHB 102155-sp.	13	AF518664	AF518593	AF518698 (as mtLSU)	n/a
Uthatobasidium sp.	FO 30284	8	AJ406434	n/a	n/a	n/a
BOTRYOBASIDIUM						
botryosum	FCUG 1750 = AFTOL 604	3	DQ089013	AY662667	n/a	n/a
botryosum	KHL 11081	14	AY586638	n/a	n/a	n/a
candicans	GEL 3083	8	AJ406440	n/a	n/a	n/a
candicans	GEL 2090	8	AJ406441	n/a	n/a	n/a
conspersum	PBM 2747 = AFTOL 1766	3	AFTOL	n/a	n/a	n/a
conspersum	KHL 11063	14	AY586657	n/a	n/a	n/a
grandisporum	FO 40862	8	n/a	n/a	AJ389798	n/a
isabellinum	GEL 2109	7, 10, 11	AF393047	AF026610	AF026652	AY218475
isabellinum	GEL 2108	8	AJ406438	n/a	AJ389799	n/a
obtusisporum	GEL 3030	1	DQ898729	DQ898739	DQ898733	DQ898769
simile	GEL 2348	1	DQ898730	DQ898740	DQ898734	DQ898770
sp.	GEL 4968	8	AJ406444	n/a	n/a	n/a
sp.	GEL 5132	8	AJ406445	n/a	n/a	n/a
subcoronatum	FCUG 1286 = AFTOL 614	8, 7, 3	AY647212	AY662666	AJ389801	AFTOL
subcoronatum	GEL 4673	8	AJ406442	n/a	n/a	n/a
subcoronatum	GEL 2936	8	n/a	n/a	AJ389809	n/a
vagum	GEL 4181	8	AJ406439	n/a	n/a	n/a
TULASNELLA						
asymmetrica	MAFF 305807 = AFTOL 1678	32	AFTOL	AFTOL	n/a	n/a
asymmetrica	MAFF 305806	1	DQ388046	n/a	n/a	n/a
asymmetrica	MAFF 305808	1	DQ388047	n/a	n/a	n/a
asymmetrica	MAFF 305809	1	DQ388048	n/a	n/a	n/a
calospora	MAFF 305801	1	DQ388041	n/a	n/a	n/a
calospora	MAFF 305802	1	DQ388042	n/a	n/a	n/a
calospora	MAFF 305803	1	DQ388043	n/a	n/a	n/a
calospora	MAFF 305804	1	DQ388044	n/a	n/a	n/a
calospora	MAFF 305805	1	DQ388045	n/a	n/a	n/a
eichleriana	GEL 4059	27	n/a	n/a	AF069630	n/a

SUPPLEMENTARY TABLE I. Continued

TAXA	STRAIN	SOURCE OF DATA [a]	nLSU	nSSU	mtSSU	RPB2
pruinosa	DAOM 17641 = AFTOL 610	12, 13, 3	AF518662	n/a	AF334894	AFTOL
sp.	GEL 4461	8	AJ406436	n/a	n/a	n/a
sp.	GEL 7456	8	AJ406437	n/a	n/a	n/a
sp.	FCUG 2668 = AFTOL 622	3	n/a	AFTOL	n/a	AFTOL
violea	DAOM 222001	17, 12	AY293216	n/a	AF334895	n/a
violea	GEL2561	1	n/a	n/a	DQ898735	DQ898768
violea	FCUG 125 = AFTOL 621	3	n/a	AFTOL	n/a	AFTOL
violea	MAFF305810 =AFTOL 1879	1	AFTOL	Weiß	n/a	n/a
sp. (as B. curtisii)	GEL5130	1	DQ898731	DQ898737	DQ898736	DQ898771
SEBACINALES						
Craterocolla cerasi	V. Kummer 02.12.2001	28	AY505542	n/a	n/a	n/a
Piriformospora indica	DSM 11827 = AFTOL 612	17, 3	AY293202	AY293147	AY293238	AFTOL
Piriformospora indica	n/a (India)	28	AY505557	n/a	n/a	n/a
Sebacina dimitica	MW 525	28	AF291364	n/a	n/a	n/a
Sebacina incrustans	PBM 2709 = AFTOL 1626	3	AFTOL	AFTOL	n/a	n/a
Sebacina incrustans	RoKi 946	28	AY505545	n/a	n/a	n/a
Sebacina sp.	F 1143539 = AFTOL 1516	3	AFTOL	AFTOL	n/a	n/a
Sebacina sp.	AFTOL 1517	3	AFTOL	AFTOL	n/a	n/a
Serendipita vermifera	CBS 572.83	29	AF202729	n/a	n/a	n/a
Serendipita vermifera	Warcup 768	28	AY505551	n/a	n/a	n/a
Tremellodendron pallidum	PBM 2324 = AFTOL 699	3	AY745701	AFTOL	n/a	AFTOL
Tremellodendron ocreatum	MCA 2069	30	AY393696	n/a	n/a	n/a
OUTGROUPS						
Auricularia auricula-judae	MW 446 = AFTOL 1681	32	AFTOL	AFTOL	n/a	AFTOL
Auricularia auricula-judae	FPL 11504	31	n/a	n/a	U27022	n/a
Auricularia sp.	FPL 8953 = AFTOL 528	3	AY691892	AY705954	n/a	AFTOL
Gomphus clavatus	OSC 97616 = AFTOL 725	3	AY647207	AY752968	n/a	n/a

SUPPLEMENTARY TABLE I. Continued

TAXA	STRAIN	SOURCE OF DATA [a]	nLSU	nSSU	mtSSU	RPB2
Gomphus floccosus	DSH 94-002	7, 6	AF287862	AF026637	AF026679	n/a
Ramaria rubella	PBM 2408 = AFTOL 724	3	AFTOL	AFTOL	n/a	AFTOL
Gautieria otthii	REG 636 = AFTOL 466	10, 3	AF393058	AF393043	AF393085	AFTOL

[A] Source of data
1 = this work
2 = Dunham et al 2003
3 = P. Matheny, AFTOL
4 = Dahlman et al 2000
5 = Pine et al 1999
6 = Hibbett et al 2000
7 = Hibbett et al 1997
8 = E. Langer, GenBank
9 = Larsson & Larsson 2003
10 = Binder & Hibbett 2002
11 = Wang et al 2004
12 = Hibbett & Donoghue 2001
13 = Hibbett & Binder 2002
14 = Larsson et al 2004
15 = Nilsson et al, GenBank
16 = Platas et al, GenBank
17 = Binder et al 2005
18 = Liu & Hall 2004
19 = Henkel et al 2005
20 = R. Kjoller, GenBank
21 = Thacker & Henkel 2004
22 = Moncalvo et al 2002
23 = Lutzoni 1997
24 = Gargas et al 1995
25 = Gonzalez et al 2001
26 = Janusz et al. GenBank
27 = E. Langer & G. Langer GenBank
28 = Weiß et al. 2004
29 = D.L. Taylor GenBank
30 = Henkel et al. 2004
31 = Hibbett & Donoghue 1995
32 = M. Weiβ & S. Garnica, provided to AFTOL

SUPPLEMENTARY TABLE II. Statistics for the molecular phylogenetic analyses conducted in this study

Dataset	Number of taxa	Alignment length (bp)	Number of characters		
			retained	variable	p-informative
All taxa nLSU	140	1005	459	268	173 (38%)
All taxa nSSU	75	2712	1277	554	450 (35%)
All taxa mtSSU	62	926	423	269	188 (44%)
All taxa RPB2	46	1075	796	451	409 (51%)
All taxa combined	34	5718	2955	1307	995 (33%)
Cantharellus combined	19*	4259	4259	402	165 (4%)
Sistotrema s.l. nLSU	60	907	907	330	206 (23%)
Tulasnella nLSU	15	859	711	301	241 (34%)
Cerato+Botryo nLSU	22	882	882	215	170 (19%)

rellus only, all genes combined (19 strains; each strain had a nLSU sequence, 11 had a nSSU sequence, 13 had a mtSSU sequence and five had a RPB2 sequence; see SUPPLEMENTARY TABLE I); *Sistotrema sensu lato*, nLSU data only; *Botryobasidium*-Ceratobasidiaceae, nLSU only; and *Tulasnella*, nLSU only.

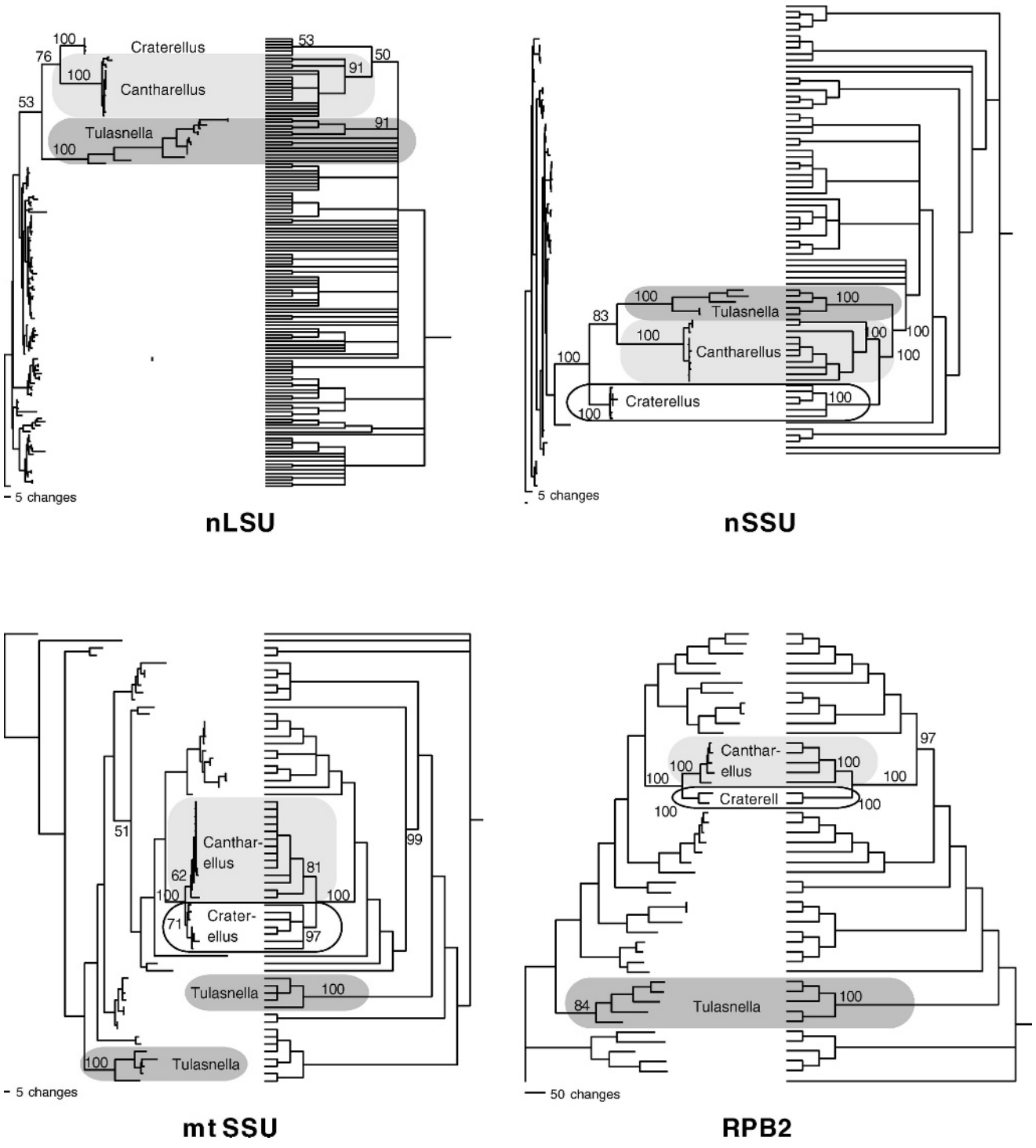
We attempted to reconstruct a ''supertree'' to bring together the separate (but optimized) analyses into a single phylogenetic tree, as described in Sanderson et al (1998). Only strongly supported nodes (pp greater or equal to 0.95) were scored to create the matrix representation submitted to maximum-parsimony analysis for a supertree reconstruction.

RESULTS

Statistics for the data matrices used in this study are presented (SUPPLEMENTARY TABLE II).

Analyses of the all-taxa nLSU dataset.—One hundred forty nLSU sequences were aligned at 1005 positions, of which 546 were discarded because homology inference of sequences from *Craterellus, Cantharellus* and *Tulasnella* with those of the other taxa was problematic. Topologies of both the MPB and B-MCMC trees indicated monophyly of *Craterellus* (100% bootstrap support [bs] and a Bayesian posterior probability [pp] of 0.53, respectively, noted hereafter as bs/pp), *Cantharellus* (100/0.91), *Multiclavula* (41/0.62), *Hydnum* (65/0.98), Ceratobasidiaceae (77/0.53), *Botryobasidium* (84/0.96), Ramariaceae-Gomphaceae (85/0.94), and Sebacinales (98/0.95). *Tulasnella* was monophyletic in the MPB tree (100% bs) but collapsed in the B-MCMC tree. Neither tree retrieved *Clavulina* and *Sistotrema* as monophyletic groups although there was no strong evidence against their monophyly. The evolutionary relationships of *Sistotrema* species remained largely unresolved, except for two relatively well supported clades respectively composed of *S. eximum, S. sernanderi, S. biggsiae, S. octosporum* and *S. efibulatum* (72/1.00; referred thereafter as the *S. eximum* group), and of *S. adnatum* and *S. coronilla* (87/0.89). Deeper nodes generally were resolved poorly, but both trees grouped together all members of *Tulasnella, Multiclavula, Hydnum, Clavulina, Sistotrema, Craterellus* and *Cantharellus* (18/0.50). A sister group relationship of *Craterellus* and *Cantharellus* was weakly suggested in both analyses (75/0.50), and the MPB tree weakly suggested (53% bs) a possible close relationship of these genera with *Tulasnella*. However these three genera are on significantly long branches with respect to all the other taxa included in the analyses (SUPPLEMENTARY FIG. 1).

Analyses of the all-taxa nSSU dataset.—Seventy-five nSSU sequences were aligned in 2712 positions. After removal of intron regions and ambiguously aligned characters (the latter again largely due to the inclusion of sequences representing *Craterellus*, *Cantharellus* and *Tulasnella*), 1277 characters were retained in the analyses. Both the MPB and B-MCMC analyses strongly supported monophyly of *Craterellus* (100/1.00), *Cantharellus* (100/1.00), *Tulasnella* (100/1.00), *Multiclavula* (100/1.00), Ramariaceae-Gomphaceae (100/1.00), *Botryobasidium* (97/1.00) and Sebacinales (75/0.99). The two analyses produced weak support for monophyly of *Clavulina* (55/0.73) and *Hydnum* (23/0.65) and resulted in conflicting topologies for Ceratobasidiaceae (31/polyphyletic). As in the nLSU analyses the evolutionary relationships of *Sistotrema* species largely were unresolved, except for support of the monophyly of the *S. eximum* group (68/0.99). The two samples of *S. raduloides* that clustered together in the nLSU analyses (69/1.00) were placed in separate, unresolved positions in the nSSU analyses. At deeper nodes there were strong supports in both analyses for monophyly of *Multiclavula, Hydnum, Clavulina, Sistotrema, Craterellus, Cantharellus* and *Tulasnella* (76/1.00) as well as for monophyly of the latter three



SUPPLEMENTARY FIG. 1. Tree topologies showing the conflicting placements of *Tulasnella, Cantharellus* and *Craterellus* both among genes and depending on the reconstruction method used. For each gene, the tree on the left is a 50% majority-rule parsimony-bootstrap tree (branch lengths are shown to depict among-taxa sequence variation), while the tree on the right is a 50% majority rule consensus of the trees sampled with Bayesian Markov chain Monte Carlo analyses. Values associated to branches show bootstrap statistical supports and Bayesian posterior probabilities, respectively, for some nodes of interest.

genera (100/1.00), in agreement with nLSU data. However in the MPB tree *Tulasnella* and *Cantharellus* were well supported as sister groups (83% bs) whereas in the B-MCMC tree *Craterellus* and *Cantharellus* were strongly supported as sister groups (pp = 1.00). These conflicting relationships are depicted (SUPPLEMENTARY FIG 1, which clearly shows that for both nSSU and nLSU data the latter three genera are on significantly longer branches than are the other taxa).

Analyses of the all-taxa mtSSU dataset.—The mtSSU dataset included 67 taxa (SUPPLEMENTARY TABLE I). There were no major problems in sequence alignment after removal of an intron region near the 5′ end and the discarding of poor sequences at the 5′ and 3′ ends. Both MPB and B-MCMC analyses moderately to strongly supported monophyly of *Craterellus* and *Hydnum* (71/0.97), *Cantharellus* (62/0.81), *Tulasnella* (97/1.00), *Botryobasidium* (97/0.98), Ceratobasidiaceae (100/1.00) and Ramariaceae-Gomphaceae (100/1.00). Similar to nLSU and nSSU analyses, *Sistotrema* was not monophyletic. However, in agreement with both nLSU and nSSU data, there was support for monophyly of *S. eximum, S. sernanderi, S. biggsiae, S. octosporum* and *S. efibulatum* (98/0.98). Also in agreement with nSSU data, but contrary to nLSU data, the two samples identified as *S. raduloides* did not cluster together. mtSSU sequence analyses also suggested a relationship between *Clavulina* (represented here by only two species) and the *S. brinkmannii-oblongisporum-resinicystidium* clade (96/0.95). This relationship was not resolved in the nLSU and nSSU gene trees. *Multiclavula*, represented here by a single sequence, strongly clustered with the latter clade in the B-MCMC analysis (pp = 0.99) but stood in an isolated position in the MPB analysis. At a deeper node, there was a weak support for monophyly of a clade including *Multiclavula, Hydnum, Clavulina, Sistotrema, Craterellus* and *Cantharellus* (41/0.73). *Tulasnella* was monophyletic (97/1.00) and sister group of the above clade in the B-MCMC analysis (pp = 0.89) but not in the MPB analysis. The placement of *Tulasnella* by mtSSU data is in sharp conflict with its placement from nLSU and nSSU data (SUPPLEMENTARY FIG. 1). There was also no indication from mtSSU data that Sebacinales is more closely related to the cantharelloid taxa than to Ramariaceae-Gomphaceae. The topology of the mtSSU gene tree indicates that this dataset was not plagued by problems relating to heterogeneous rates of molecular evolution and long-branch attraction (SUPPLEMENTARY FIG. 1).

Analyses of the all-taxa RPB2 dataset.—The RPB2 sequence alignment for 46 cantharelloid taxa was largely unambiguous. Both MPB and B-MCMC analyses resulted in trees that were more resolved than those obtained from the ribosomal DNA datasets. There was strong support for a sister group relationship between *Craterellus* and *Cantharellus* as well as their respective monophyly (all 100/1.00). *Hydnum* was monophyletic (100/1.00) and sister group of *S. confluens* (100/1.00). Samples of *S. brinkmannii, S. oblongisporum, S. farinaceum* and *S. resinicystidium* form a monophyletic groups (100/1.00), with the inclusion of our single representative of *Clavulina* (the latter being strongly nested within *Sistotrema* species in the B-MCMC analysis). All the above taxa, with the addition of *S. raduloides, S. adnatum* and *S. coronilla* (the latter two clustering together, 100/1.00), formed a weakly supported clade (9/0.62) in a more derived position. Further down the RPB2 trees *S. eximum* and *S. octosporum* were monophyletic (85/1.00) and sister of *S. athelioides* in the MPB but not in the B-MCMC tree. There was strong support for monophyly of *Botryobasidium* (100/1.00) and *Tulasnella* (84/1.00), with the latter being in a more basal position than the other cantharelloid taxa. At the base of the trees (rooted with *Dacrymyces*), Sebacinales (monophyletic, 100/1.00) and Ramariaceae-Gomphaceae (monophyletic, 100/1.00) form a cluster with *Auricularia* (48/0.99). Similarly to mtSSU there was no apparent long-branch attraction problem in the RPB2 dataset and no indication that *Tulasnella* might be evolutionary closely related to the *Cantharellus-Craterellus* clade (SUPPLEMENTARY FIG. 1).

Combined analyses of the all-taxa four-gene dataset.—The combined four-gene matrix consisted of 37 taxa and 2955 unambiguously aligned characters, of which 1307 were variable and 995 were parsimony informative (SUPPLEMENTARY TABLE II). The ILD test indicated that the four-data partition was incongruent ($P < 0.01$), in agreement with empirical observation of the tree topologies produced from the different data partitions (SUPPLEMENTARY FIG. 1), which indicate strong conflicts in the placement of *Tulasnella*. The problematic placement of *Tulasnella* was confirmed further in analyses that showed incongruent placement of members of this genus depending on the data included in the analyses. For instance, when *Tulasnella* sp. GEL5130 (which has data for all genes), *T. asymmetrica* MAFF305807 (nLSU and nSSU data only) and *T. violea* GEL2561 (RPB2 and mtSSU data only) were included in the analyses, *Tulasnella* was monophyletic (81/1.00) and sister of the *Cantharellus-Craterellus* clade (53/0.82). In contrast, when *Tulasnella* sp. GEL5130 was excluded from the analyses, then *Tulasnella* was not monophyletic; *T. asymmetrica* strongly clustered with the

chanterelles (100/1.00), whereas *T. violea* stood alone in a more basal position in the tree. *Tulasnella* therefore was removed from the combined data matrix. After removal of *Tulasnella*, the ILD test showed congruence between the RPB2 and mtSSU data partition (P = 0.17) and between the nLSU and nSSU partitions (P = 0.15), but not between all partitions (P = 0.01) unless *Cantharellus* and *Craterellus* were removed (P = 0.07). However, because we did not detect significantly supported topological conflicts in the placement of these two genera in the separate analyses, we kept them in the combined analyses. All but five nodes received > 0.95 pp and many also had bootstrap support > 50% (FIG. 1A). The only topological differences between the MPB and B-MCMC trees involved nodes with less than 0.95 pp and bs no greater than 65%, with one exception; *Hydnum* and *S. confluens* were monophyletic in the MPB tree (88% bs) and paraphyletic in the B-MCMC tree (pp = 1.00). At the base of the tree (rooted with *Dacrymyces*) the Sebacinales was monophyletic (100/1.00) and sister group (41/1.00) of *Gautieria* (Ramariaceae)-*Auricularia* (monophyletic, 36/1.00). *Ceratobasidium* sp. GEL 5602 (the only Ceratobasidiaceae represented in this analysis) is the next derived taxon. Monophyly of all other included taxa is moderately supported (47/0.96), but there is strong support for a monophyletic *Botryobasidium* (100/1.00) as a sister group of all the other taxa (95/1.00). Following up the tree, *S. eximum* and *S. octosporum* clustered together (100/1.00), then comes *S. athelioides* and *S. adnatum*, either mono- (MPB tree, 19% bs) or paraphyletic (B-MCMC tree, pp = 0.91). Finally, in a more derived position a clade composed of *Craterellus, Cantharellus, Hydnum* and *S. confluens* (53/1.00), is the sister group (65/1.00) of a clade that included the remaining *Sistotrema* species, *Clavulina* and *Multiclavula* (53/1.00). The position of *S. raduloides* remained unresolved.

Analyses of the four-gene dataset for Cantharellus.—Combined analyses of nLSU, nSSU, mtSSU and RPB2 sequences for 19 *Cantharellus* strains were conducted. All strains had a nLSU sequence, but nSSU, mtSSU and RPB2 data were missing respectively for six, five and 14 strains (SUPPLEMENTARY TABLE I). All positions could be aligned unambiguously and yielded 402 variable and 165 parsimony informative characters. The B-MCMC and MPB analyses resulted in identical tree topologies with most nodes receiving high statistical supports (FIG. 1C). *Cantharellus* was divided in two major groups, the *C. cibarius* group *sensu lato*, in which *C. subalbidus, C. cascadensis, C. formosus, C. persicinus* and *C. lateritius* were distinguished from the *C. cibarius* complex *sensu stricto*, and the *C. cinnabarinus-minor-appalachiensis* group.

Analyses of nLSU data in Sistotrema *and allies (excluding* Cantharellus-Craterellus*).*—Results from the all-taxa four-gene analyses (FIG. 1A) indicated the presence of a strongly supported clade (100/1.00) consisting of representative isolates of *Sistotrema, Pneumatospora (=Tricellulortus), Clavulina, Multiclavula, Hydnum, Craterellus* and *Cantharellus*. After removal of the latter two genera from the nLSU data matrix because of alignment difficulties (see above), all positions could be unambiguously aligned and yielded 330 variable and 206 parsimony informative characters. Results from phylogenetic analyses are provided (FIG. 1B). In contrast to the nLSU analyses that included all the taxa and about half the number of included characters, *Clavulina* was recovered as a monophyletic genus, sister of *Membranomyces* (100/0.83). In agreement with the all-taxa four-gene analysis (FIG. 1A), they are part of a larger clade that also includes *Multiclavula* and the *S. brinkmannii-oblongisporum* group. Also in agreement with earlier analyses *Sistotrema* was not monophyletic. *S. muscicola, S. confluens* and *S. alboluteum* clustered with *Hydnum* (100/0.95), *S. raduloides* and *S. athelioides* stood in unresolved position, and the remaining species formed a moderate to weakly supported (98/0.33) group with the anamorphic genus *Pneumatospora*.

Analyses of nLSU data for Botryobasidium *and Ceratobasidiaceae.*—nLSU sequence alignment among our sampling of *Botryobasidium* and Ceratobasidiaceae species was unambiguous in all positions and yielded 215 variable and 170 parsimony informative characters. Within *Botryobasidium* the tree topology indicated that species circumscription in *B. candicans, B. botryosum* and *B. simile* is still unclear (FIG. 1D). Similarly in the Ceratobasidiaceae the identity of the strain labeled *Uthatobasidium* sp. F030284 needs further scrutiny.

Analyses of nLSU data for Tulasnella.—The nLSU sequence alignment among the 15 *Tulasnella* isolates sampled was 859 bp in length and ambiguous in 148 positions, which further highlights the high rate of rDNA evolution in this genus. In addition a considerable amount of sequence divergence was observed among strains identified as *T. asymmetrica* and *T. calospora,* respectively, suggesting that these names encompass large species complexes. Difficulties in circumscribing and identifying *Tulasnella* species (or the inability of nLSU sequences to do so) also were shown in the polyphyly of our two samples originally identified as *T. violea* (FIG. 1E).

LITERATURE CITED ONLY IN THE SUPPLEMENTARY MATERIAL

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