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

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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-

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 (Sᴜᴘᴘʟᴇᴍᴇɴᴛᴀʀʏ Tᴀʙʟᴇ 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 supplement.)

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
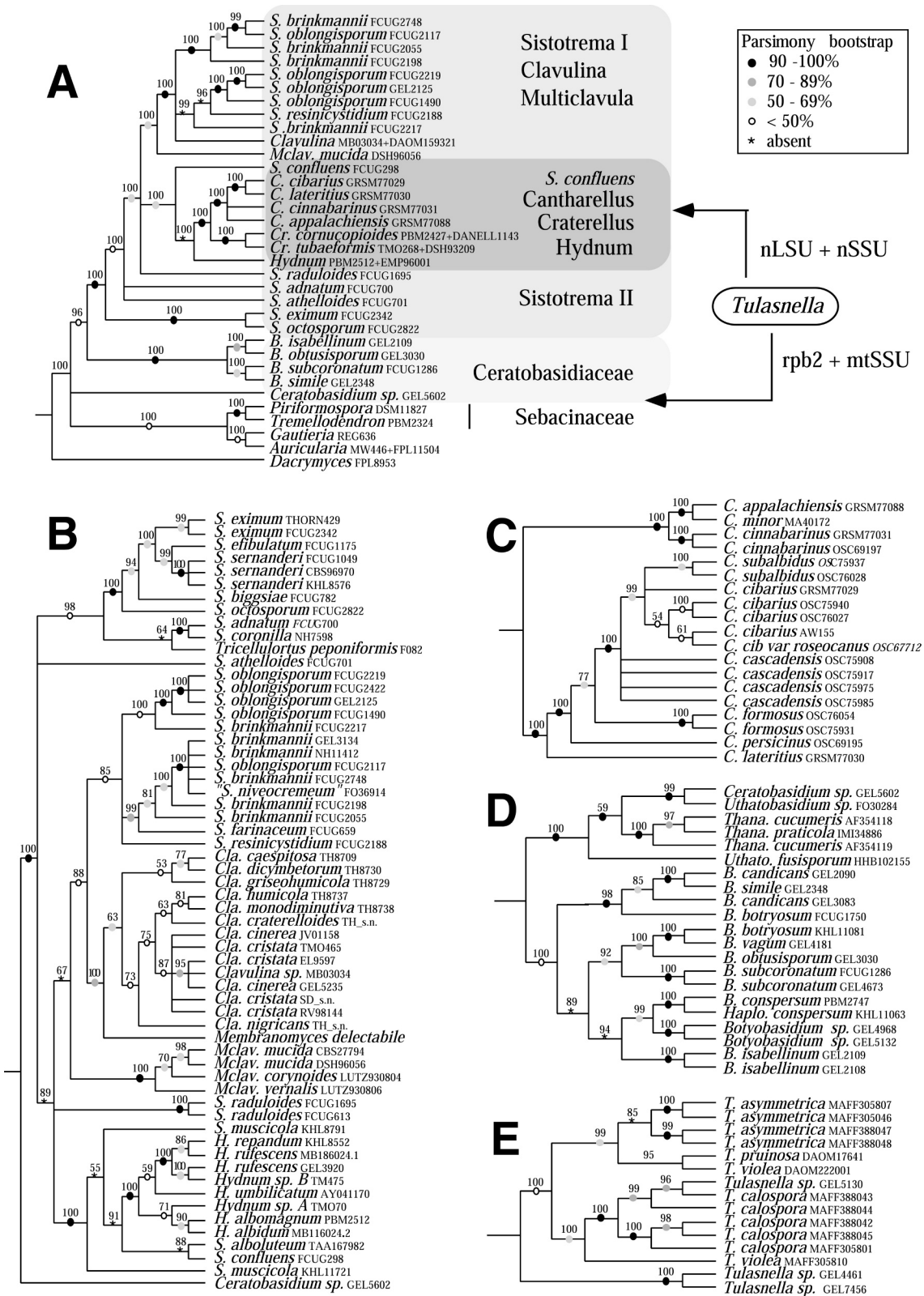
FIG. 1. Inferred phylogenetic relationships for: A. the all-taxa (minus *Tulasnella*) four-gene analyses showing the ambiguous placement of *Tulasnella* depending on which genes are analyzed; B. nLSU analyses in *Sistotrema* and close allies (minus *Cantharellus* and *Craterellus*); C. four-genes analyses 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 irpicioid-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 (*Ceratobasi-dium, 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 SUPPLE-MENT). 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 Ceratobasi-diaceae. Such placement also agrees with the non-monophyly 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 *Tulas-nella*, 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, Pirifor-mospora* 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 Sebaci-nales (*Piriformospora indica* and *Tremellodendron pallidum*) strongly clustered with *Gautieria* (representing the gomphoid-phalloid clade) and *Auricu-laria* (a traditional heterobasidiomycete) when the tree is rooted with *Dacrymyces* (heterobasidiomy-cetes). 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 hyme-nomycete lineage composed of morphologically and ecologically diverse fungi (FIG. 2). A possible synapo-morphy 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 Cerato-basidiaceae 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 homo-basidiomycetes, 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 im-perforate parenthesomes have been found in *Cantharellus* and *Botryobasidium*, but perforate par-enthesomes have been reported from Ceratobasi-diales (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 par-enthesome type unites members of the cantharelloid clade.

This was the first study using sequence data from a protein-coding gene (RPB2) for molecular system-atics 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 (SUPPLEMENTA-RY FIG. 1) and better resolves phylogenetic relation-ships 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.
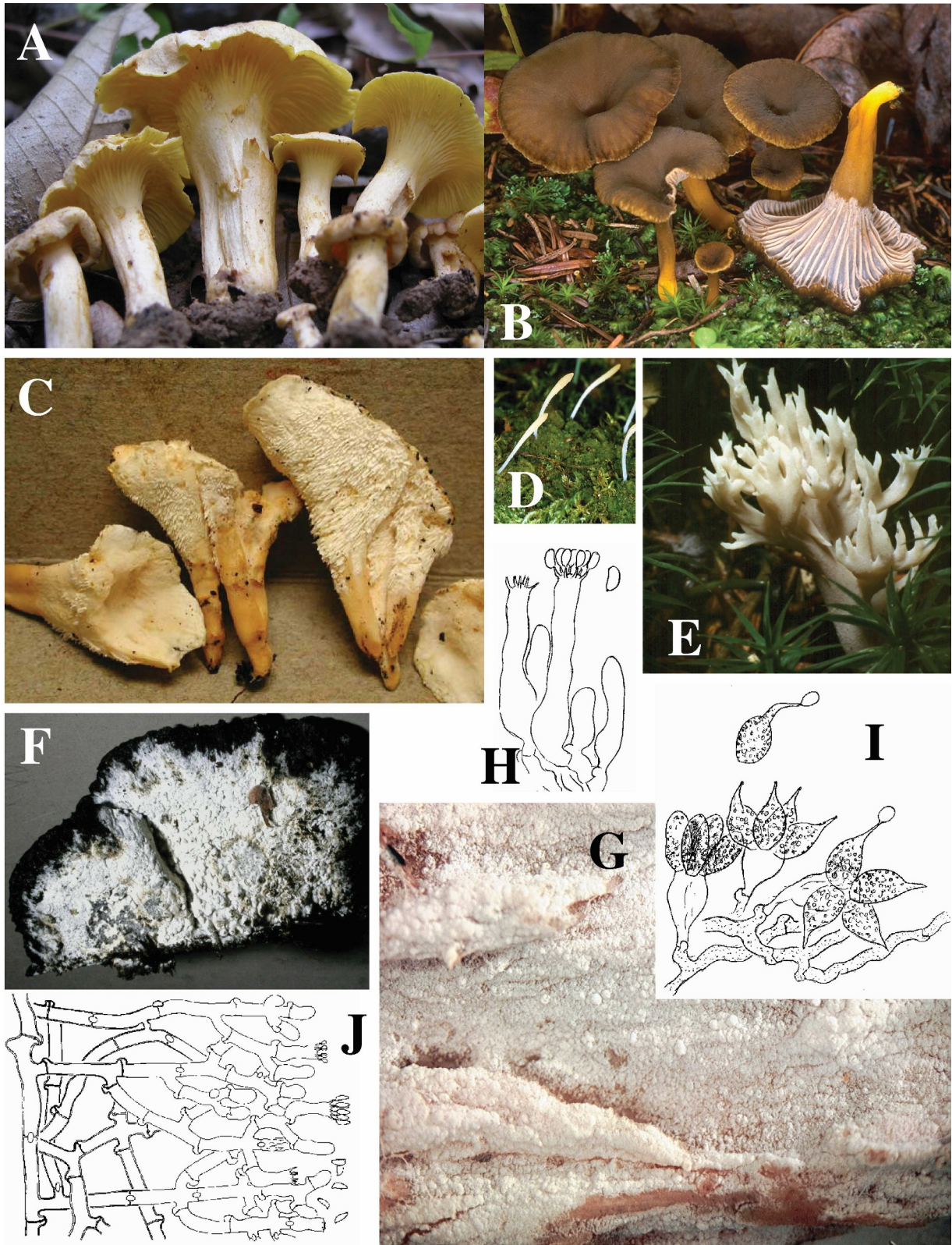
FIG. 2. Morphological diversity in the cantharelloid clade. Basidiocarps of: A. *Cantharellus* aff. *cibarius* (image from J.-M. Moncalvo); B. *Craterellus tubaeformis* (M. Wood); C. *Sistotrema confluens* (R. Halling); D. *Multiclavula mucida* (M. Wood); E. *Clavulina cinerea* (E. Langer); F. *Botryobasidium 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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