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Phylogenetic relationships of cantharelloid and clavarioid Homobasidiomycetes based on mitochondrial and nuclear rDNA sequences

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Abstract: Sequence data from mitochondrial and nuclear small subunit rDNA were used to estimate phylogenetic relationships of cantharelloid and clavarioid Homobasidiomycetes. Sixty-five diverse Homobasidiomycete species were investigated, including 23 cantharelloid and clavarioid species. Although nodes deep in the tree could not be resolved, four lineages containing cantharelloid and clavarioid fungi were identified. (i) Cantharellaceae (*Cantharellus, Craterellus*) is closely related to *Hydnum,* which is toothed, *Stichoclavaria,* which is a simple club, and *Clavulina,* which is coralloid. These taxa all have stichic nuclear division, which is a synapomorphy supporting this clade. (ii) *Clavariadelphus* is closely related to *Gomphus* and *Ramaria.* This relationship is supported by green reactions of sporocarps treated with iron salts, which is reflective of the presence of the compound pistillarin. The nearest relatives of these cantharelloid and clavarioid fungi are gasteromycetes, including the earth star *Geastrum,* the stinkhorn *Pseudocolus,* and the "cannon-ball fungus" *Sphaerobolus.* (iii) The clavarioid fungi *Clavaria, Clavulinopsis, Pterula,* and *Typhula* appear to be derived from the lineage that contains most of the gilled fungi. (iv) *Clavicorona* is closely related to *Auriscalpium,* which is toothed, and *Lentinellus,* which is gilled. This lineage is united by amyloid spore ornamentation. Although these results suggest that there has been extensive convergence in fruiting body morphology, certain anatomical and biochemical features appear to be phylogenetically informative, notably stichic nuclear division, presence of pistillarin, and cyanophily or amyloidity of spore ornamentation.

Key Words: Cantharellaceae, Clavariaceae, evolution, fungi, Gomphaceae, phylogeny, ribosomal DNA, systematics

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INTRODUCTION

Fruiting bodies of cantharelloid and clavarioid Homobasidiomycetes include funnel-shaped or pileate sporocarps with smooth, wrinkled, or lamellate hymenophores, and unbranched club or branched coralloid sporocarps with smooth or folded hymenophores. Ecological habits range from saprophytism and parasitism to ectomycorrhizal and lichenized mutualisms. Anatomical and biochemical diversity is found in characters such as spore ornamentation and reactivity, hyphal structure, patterns of meiotic division, secondary compounds, and chemical structure of pigments (TABLE I).

Although all modern authors agree that the cantharelloid and clavarioid fungi are polyphyletic (e.g., 2, 4, 10, 11, 12, 14, 19, 21, 24, 43, 58, 72, 74, 83), evolutionary relationships of monophyletic taxa have not been resolved. Relatively few morphological characters have been identified that can be compared across genera, and many of these support incongruent relationships. Various authors have emphasized different suites of characters and consequently have proposed conflicting evolutionary histories (e.g., 10, 12, 14 vs 19 vs 72). A preliminary phylogenetic analysis using published morphological characters failed to resolve relationships among genera of cantharelloid and clavarioid fungi (EM Pine unpubl). Results presented here use DNA sequence data as an independent and abundant source of characters for comparisons across diverse lineages.

This discussion treats only taxa and characters relevant to results of this study. Corner (10, 12, 14), Donk (19), and Petersen (74) provide broad taxonomic reviews of cantharelloid and clavarioid fungi. Selected authors' taxonomic treatments of key genera are summarized (see TABLE II).

Cantharelloid and clavarioid fungi figure prominently in hypotheses about the origin of the fleshy basidiomycetes (12, 15, 31, 32, 43, 58, 72, 83, 84). Their fruiting forms can be arranged in a transformation series, with simple clubs at one extreme, cantharelloid forms intermediate, and agaric forms at the other extreme. Corner (15) proposed the "*Cla-*

varia theory" of basidiomycete evolution, which treats the cantharelloid and clavarioid fungi as a basal paraphyletic group from which all other Homobasidiomycetes have been derived. Corner suggested that the simple club with a smooth hymenophore (e.g., *Clavaria*) is the ancestral state of the fleshy fungi, from which have been derived first club-shaped and cantharelloid intermediates with folded hymenophores (e.g., *Clavariadelphus* and *Cantharellus,* whose hymenial configurations differ from true lamellae in the orientation of hyphae in the trama), and eventually gilled mushrooms. Corner's model has had a strong influence on subsequent evolutionary hypotheses. For example, Jülich (43) suggested that Clavariaceae was derived from the Auriculariales (jelly fungi) or their ancestors, and that Cantharellales is the basal group of Homobasidiomycetes. Miller and Watling (58 p 439) state that "the logical extension from the clavarioid condition among epigeous taxa is the cantharelloid basidiome," and suggest that agarics have been derived multiple times from cantharelloid ancestors. Other authors agree that there must have been transformations among coralloid, cantharelloid, and agaric forms, but propose the opposite polarity, suggesting that lineages containing cantharelloid, coral, and club fungi have been derived from agaric ancestors (2, 30, 72, 83).

The agarics *Hygrocybe* and *Gerronema* have been suggested as close relatives of Cantharellaceae. *Hygrocybe* is similar to *Cantharellus* in having thick, waxy, decurrent gills, bright orange and yellow pigments, long and narrow basidia, and hyaline, unornamented, non-reactive spores (34). *Gerronema* (= *Omphalina*) *chrysophyllum* resembles members of Cantharellaceae in spore color, hymenial anatomy, basidiocarp color, general aspect, and molecular structure of carotenoid pigments (2, 29, 48, 83). Yet chanterelles depart from true mushrooms in several important characters (TABLE I), including anatomical differences between cantharelloid gill-folds and true agaric gills (12 p 19), leading several authors to ascribe similarities between Cantharellaceae and *Hygrocybe* or *Gerronema* to convergent evolution (12, 19 p 245, 33).

Singer (83 p 126) suggested that "A further 'bridge' between Aphyllophorales and Agaricales might be seen in *Linderomyces,*" a genus with a bilateral trama (true gills) and unusual "cocsinoid" (sieve-like) hyphae (82), but with microscopic features and chemical reactions characteristic of Gomphaceae (13, 69, 71). Singer originally placed *Linderomyces* in Paxillaceae (82); he later concluded that the genus represented an independent origin of gills within Gomphaceae, but thought it might be "a starting point for an evolution which would lead from the Gomphaceae to the Paxillaceae" (83 p 126). Petersen (69) concluded that *Linderomyces* was a synonym for *Gloeocantharellus,* a gomphaceous genus whose morphology has been described as intermediate between *Cantharellus* and *Gomphus* (81). Corner (12, 15) and Petersen (71, 72) agreed with Singer that *Gloeocantharellus/Linderomyces* belongs in Gomphaceae, but thought it could represent an evolutionary link with Paxillaceae and some Boletaceae.

Anatomical features suggest that cantharelloid and clavarioid fungi comprise several independent lineages (TABLE I). Spore morphology can be used to delineate three groups. Hyaline, unornamented spores that do not react to Meltzer's reagent or Cotton Blue are characteristic of most of the known cantharelloid and clavarioid fungi. Spores with distinctive amyloid ornamentation are found in the coralloid genus *Clavicorona*; Donk (19) used this feature, along with presence of gloeocystidia, to transfer *Clavicorona* from Clavariaceae to Hericiaceae. The remaining spore type, ochraceous with cyanophilic ornamentation, is found in genera with a variety of fruiting body forms: *Gomphus* (cantharelloid), *Ramaria* (coralloid), *Beenakia* (hydnoid), *Kavinia* and *Ramaricium* (resupinate), and *Gloeocantharellus* (= *Linderomyces*) (agaric). Despite their macromorphological diversity, these taxa have been grouped in Gomphaceae (18, 19, 21, 44, 46, 52, 71, 83), a placement that is supported by shared green reactivity of fruiting bodies treated with $FeSO_4$. The club-shaped genus *Clavariadelphus* also reacts with ferric salts, but has smooth, hyaline, unornamented spores (56). If macrochemical reactions and mode of nuclear division are emphasized, *Clavariadelphus* is placed with Gomphaceae (2, 19, 30, 32, 56, 72, 90), but emphasis on spore morphology supports a relationship with Cantharellaceae or *Clavaria* (10 p 25, 11, 12, 14, 58, 65, 68, 72).

The position and orientation of the first nuclear division of meiosis has been proposed as a taxonomically important character (19, 41, 42, 55). In most Homobasidiomycetes that have been examined, division takes place near the apex of the basidium with the meiotic spindle transverse to the long axis of the basidium (6 p 267, 39, 42, 55, 63). This pattern is called chiastic division (see FIG. 3). In contrast, in *Cantharellus* (39, 42, 55), *Craterellus* (42, 55), *Clavulina* (42, 55, 63), *Stichoclavaria* (= *Multiclavula*) (39, 42), *Clavulicium* (6 p 267), *Sistotrema* (6 p 267, 49), and *Hydnum* s. s. (55, 63, 80), division is near the middle of the basidium, with the spindle axis more or less parallel to the basidial axis. This pattern is called stichic division (see FIG. 3). Meiotic division can be observed only in fresh, mature fruiting bodies, and has not been examined in many taxa.

TABLE I. Selected characters of cantharelloid and clavarioid fungi and putative relatives included in this study[a]

	Basidiocarp morphology[b]	Hymenium			Habit[f]	Spores			
		Discontinuous[c]	Configuration[d]	Thickening[e]		Ornamentation[g]	Pigmentation[h]	Reaction[i]	Number[j]
Cantharellus	pi/ca (12)	+ (19)	wr/la (12)	+ (12)	em (8)	− (11, 12)	hy/(pi, ye) (12)	− (11, 12, 65)	2–8 (11, 12)
Clavaria	cl/(co) (14)	− (10)	sm	+/− (10)	te (10)	−/(+) (14, 72)	hy/(pi[f]) (10)	− (46, 65)	4 (10)
Clavariadelphus	cl/ca (56)	−/(+) (56)	sm/wr (56)	+ (56)	em	− (56)	hy/(bu) (56)	− (56, 65)	4/(1–3) (56)
Clavicorona pyxidata	co (22)	+ (10, 22)	sm	− (10)	wo (19)	− (22)	hy (22)	am (19, 51)	4 (22)
Clavulina	cl/co (10)	−/(+) (10)	sm	+ (10)	em (8)	− (10)	hy (10)	− (46)	2/(1) (10)
Clavulinopsis fusiformis[s]	cl/co (78)	− (10)	sm (72)	+ (68)	te (68)	− (68)	hy (67)	− (46, 65)	2–4 (68)
Craterellus	ca (12)	+ (11)	sm/wr (11)	+/(−) (12)	em	− (11)	hy (10)	− (11)	2–6 (12)
Gerronema	pi (83)	+	la (83)	− (11)	li/sa (83)	− (83)	hy/or (83)	− (83)	4 (83)
Gloeocantharellus	pi (71)	+ (13)	la (71)	−/(+) (13)	te (12)	wa (71, 81)	oc (70, 81)	cy (67, 70)	2–4 (13)
Gomphus	ca (12)	+ (56)	sm/wr (56)	+ (13, 70)	em (8)	wa/ri (71)	oc (56)	cy (67)	2–4/(8) (12)
Hydnum	pi (19)	+	to (19)		em	− (19)	hy (19)	− (19)	2–6 (19)
Hygrophoraceae	pi (83)	+	la (83)	− (11)	em/sa (83)	− (83)	hy (34)	− (83)	2 or 4 (83)
Lentaria s.s.[t]	co (67)	+/− (10)	sm	+ (10)	sa (67)	− (19)	hy (19, 67)		2–8 (10)
Macrotyphula[u]	cl (3, 73)	− (10)	sm (10)	+ (56)	wo (10)	− (10)	hy (10, 72)		4 (3, 10)
Pterula	cl/co (10)	+ (10)	sm	+ (10)	sa (10)	− (10)	hy (10)		2–4 (10)
Ramaria	co (10)	+ (10)	sm	+/− (10)	em (8)/sa (10)	ro/sp (74)/(−) (14)	oc (72)	cy (46, 65, 70)	1–4 (10)
Stichoclavaria[v]	cl (67)	− (10)	sm	− (67)/(+) (10)	li/(sa) (67)	− (67)	hy (67)		2–4 (67)
Typhula subg. *Typhula*	cl (3)	+ (3)	sm	− (10, 56)	sa/pa (10)	− (10)	hy (10)	− (3)	2–8 (10)

[a] Scoring is for the entire taxon listed, with rare states indicated in parentheses.

[b] Fruiting body morphology: cl = clavarioid (simple lub), co = coralloid (branching cylinder), ca = cantharelloid (funnel-shaped), pi = pileate/agaricoid.

[c] Discontinuous hymenium: − = fertile area continuous across top of basidiocarp, + = apex of fruiting body sterile (assumed true for "pi" fruiting bodies).

[d] Hymenial configuration: sm = smooth (assumed true for clavarioid and coralloid taxa unless otherwise reported), wr = wrinkled to folded, la = lamellate/appearing gilled, to = toothed/hydnoid.

[e] Thickening hymenium (sensu 10): − = hymenial layer constant thickness throughout development, + = developing basidia push past mature basidia.

[f] Habit/ecologial strategy (substrate reported only if specific ecological data not available): em = ectomycorrhizal, li = lichenized, pa = parasitic, sa = saprobic, te = terrestrial, wo = found on dead plant matter.

[g] Spore ornamentation: − = none (spores smooth), ro = roughened, wa = warty, ri = ridged or with anastomosed warts, sp = spiny/echinulate.

[h] Spore pigmentation: hy = hyaline (unpigmented), pi = pink, ye = yellow, or = orange, bu = light buff, oc = ochraceous.

[i] Reactivity of spore wall ornamentation (not including cytoplasm): − = not staining blue in Meltzer's reagent or Cotton Blue, am = amyloid, cy = cyanophilic, blank = reactivity not reported for either reagent.

[j] Number of spores (or sterigmata) per basidium.

[k] Presence/type of cystidia in hymenium: − = no cystidia of any type, le = leptocystidia/undifferentiated cystidia, gl = gloeocystidia (also known as cocsinocystidia).

TABLE I. Extended

	Hyphae				Biochemistry	
	Cystidia[k]	Skeletal[l]	Gloeoplerous[m]	Clamps[n]	Meiotic type[o]	Fe reactivity[p]	Carotenoids[q]
Cantharellus	− (11, 12)	− (12, 65)	− (11, 65)	+ (11)/(−) (13)	st (39, 42, 55)	− (19)/(lg/re [72])	+ (30)
Clavaria	− (10)	− (65)	− (65)	− (14, 72)	ch (42, 63)	vi (89)/− (72)	+[r]/− (30)
Clavariadelphus	le (56)	− (10, 65)	+ (56, 73)	+ (56)	ch (63: *Clavaria pistillaris, C. ligulus, C. truncatus*)	gr (19, 56), +pi (78)	− (29 in 30, 72)
Clavicorona pyxidata	le (10)/gl (22, 23)	− (10)	− (10)	+ (22)		− (22)	
Clavulina	−/(le) (10)	− (10)	−	+/(−) (10)	st (42: *Clavaria cinerea, C. cristata,* 55: *C. rugosa, C. grisea,* 63)		
Clavulinopsis fusiformis[s]	− (68)	− (67)	− (65)	+ (67, 68)	ch (39, 42: *Clavaria muscoides, C. subtilis,* 63)	gr, −pi (78)	− (30, 78)
Craterellus	− (11)	− (11)	− (10)	− (11)	st (42: *Cantharellus cinereus,* 55)	− (19)	+ (30)
Gerronema	−/(le) (83)	− (83)	−	+/− (83)	ch (Kühner in 39, 48)		+ (29)
Gloeocantharellus	gl (13, 81)	− (83)	+ (67, 70)	+ (71, 81)		gr (71)	− (71)
Gomphus	− (12, 70)/(le) (70)	− (12)	+ (12, 71)	+/(−) (12, 71)	ch (42: *Craterellus clavatus*)	gr, +pi (78)	− (30, 71)
Hydnum	− (19)	− (19)	−	+ (19)	st (42, 63, 80)		
Hygrophoraceae	− (83)	− (83)	−	+/− (34, 83)	ch (55)		
Lentaria s.s.[t]	− (10)	+ (65)	−	+ (72)	ch (42: *Clavaria epichnoa*)	gr (19)	
Macrotyphula[u]	− (10)	− (10)	la (56, 73)	+ (56)		− (56)	
Pterula	−/le (10)	+ (10)	−	+ (10)			
Ramaria	− (10)	+/− (16, 65)	+ (65)	+/(−) (72)	ch (42: *Clavaria abietina, C. crispula,* 63)	gr, +pi (78)	− (30, 77)
Stichoclavaria[v]	−/(le) (67)	− (10)	−	+/(−) (67)	st (39, 42: *Clavaria falcata*)		
Typhula subg. *Typhula*	− (10)	− (10)	−	+/− (10)	ch (63)		

[l] Skeletal hyphae in basidiocarp: − = absent (monomitic), + = present (dimitic).

[m] Gloeoplerous hyphae in basidiocarp: − = absent (or not reported), + = present, la = laticiferous hyphae (superficially similar to gloeoplerous hyphae, but not staining in Cotton Blue).

[n] Clamp connections throughout basidiocarp: − = absent (or a single clamp at the base of the basidium, as seen in *Clavaria*), + = present.

[o] Orientation of first meiotic nuclear division (original reports only): st = stichic (spindle oblique or parallel to long axis of basidium), ch = chiastic (spindle transverse), blank = not reported, original classification of examined specimen is listed if it was later transferred to a different genus.

[p] Reaction of fruiting body with iron salts (i.e., $FeSO_4$ or $FeCl_3$): − = no reaction, lg = light green, re = red, gr = dark green, vi = violet, +pi = compound pistillarin demonstrated to be present, −pi = pistillarin assayed but not present, blank = reactivity not reported.

[q] Carotenoid pigments in fruiting body extractions: − = absent, + = present.

[r] Characteristic of *Clavaria helicoides,* whose generic position is uncertain (see 14: p. 34–35).

[s] *C. fusiformis* has been placed variously in *Clavulinopsis, Ramariopsis,* and *Clavaria,* so scoring is limited to the single species.

[t] *Lentaria s.s.* refers to taxa remaining after the segregation of *Multiclavula* Petersen.

[u] *Macrotyphula* scoring is based largely on Corner's (10) *Clavariadelphus* subg. *Typhulopsis*; Methven (56) equates the two.

[v] *Stichoclavaria* Ulbrich is used for *Multiclavula* Petersen, see text.

Shared possession of stichic division suggests that Cantharellaceae is closely related to *Hydnum* (17, 19, 72, 74) and *Clavulina* (17, 19). If stichobasidia are deemphasized as a taxonomic character, other features suggest different relationships (TABLE I). For example, Corner (11) used hymenophore configuration, fruiting body development, clamp connections, and presence/absence of a sterile apex or pileus to split Cantharellaceae, suggesting placement of *Cantharellus* with *Clavariadelphus, Craterellus* with *Stereum,* and *Clavulina* with *Clavaria* and *Clavulinopsis*. Petersen (66) agreed that stichic *Clavulina* was related to chiastic *Clavulinopsis,* but placed *Clavariadelphus* with this complex rather than with *Cantharellus*. Reijnders and Stalpers (79) found a different pattern of hymenophore trama development in *Hydnum repandum* than in Cantharellaceae, which, combined with the absence of carotenoid pigments in *Hydnum,* led them to reject a close relationship between Cantharellaceae and *Hydnum*.

Circumscription of genera within Cantharellaceae has been controversial (74). *Craterellus* has been distinguished mainly by absence of clamp connections (4, 11, 12), but Petersen (74) noted that some species that lack clamps have been included in *Cantharellus*. Corner (11) proposed the genus *Pseudocraterellus* to contain unclamped, secondarily septate chanterelles otherwise similar to *Cantharellus*; Corner also emphasized patterns of fruiting body development, but this feature is difficult to examine and has been largely ignored by subsequent workers. Petersen (70, 74) and Bigelow (4) criticized secondary septation as a taxonomic character since it is variable among individual fruiting bodies, especially those of different ages, and is difficult to ascertain in herbarium material. Furthermore, many authors have noted cantharelloid species that exhibit combinations of features used to define different genera (e.g., *Craterellus carolinensis*) or whose placements by Corner's criteria conflict with those supported by other well-accepted characters (4, 11, 13, 70, 75). Corner himself (13) pointed out that *Cantharellus inathinus* and *C. subcibarius* can be clamped and secondarily septate; his description of *C. cuticulatus,* which is "so very obviously a *Cantharellus*" (p 786) led him to conclude that "secondarily septate hyphae without clamps, such as characterize *Pseudocraterellus,* occur in this species of *Cantharellus*" (p 785). Despite examination of pigment structure (30), spore wall anatomy (45), secondary septation, fruiting body ontogeny, and hyphal anatomy (11, 12, 13), no synapomorphies have been recognized that unambiguously distinguish *Craterellus, Cantharellus,* and *Pseudocraterellus*. Although these difficulties have led some authors to collapse all the species of Cantharellaceae into one genus (e.g., 50), or to segregate *Craterellus* into its own family (e.g., 43), such changes in taxonomic rank have not clarified relationships among cantharelloid lineages.

Clavarioid basidiomycetes are a heterogeneous group whose phylogenetic relationships have also proved extremely difficult to resolve. A few genera, such as *Clavicorona* and *Ramaria,* share distinctive features with other lineages of Homobasidiomycetes and have been removed from Clavariaceae (18, 19). Other species have autapomorphic features that have allowed segregation of the umbrella *Clavaria* into distinct genera. For example, *Clavulina* is characterized by secondarily-septate basidia with two strongly incurved sterigmata, *Pterula* by a dimitic hyphal system, and *Typhula* by the formation of sclerotia (10, 14). But such characters do not suggest higher-level relationships, and although some authors have promoted these genera to segregate families (19, 43), their nearest relatives have not been identified. Stichic division (found in *Stichoclavaria* and *Clavulina*) and carotenoid pigmentation (found in *Clavaria* subg. *Clavulinopsis* sensu Petersen, 77), link some genera to other lineages of Homobasidiomycetes (TABLE I), but these characters have not been widely accepted as synapomorphies. Thus Clavariaceae is still a polyphyletic group that is defined largely by the absence of distinguishing features.

MATERIALS AND METHODS

Twenty-five cantharelloid and clavarioid exemplars were selected to represent 23 species in 12 genera and 8 families sensu Corner (12, 14). Taxa were chosen to emphasize taxonomically controversial traits (e.g., stichic nuclear division, spore ornamentation, $FeSO_4$ reactivity), with an effort to include multiple species of each genus of chanterelles and several clavarioid genera (TABLE II). Sequences for *Clavicorona pyxidata* had been published previously (35).

Because higher-level evolutionary relationships of cantharelloid and clavarioid fungi are controversial, a broad sampling of other Homobasidiomycetes was imperative. Four taxa were chosen to represent proposed relatives of Cantharellaceae: *Hydnum repandum, Gerronema chrysophyllum,* and two species of Hygrophoraceae. *Gloeocantharellus purpurascens,* with true gills, was the sole representative of noncantharelloid or clavarioid Gomphaceae. Sequences for additional taxa were available from published and ongoing studies of Homobasidiomycete relationships (35, 36, 37). Thirty-six exemplars were selected to represent traditional families of basidiomycetes as well as unclassified lineages identified by previous phylogenetic analyses. In total, 21 families sensu Donk (19) and Singer (83) were represented.

DNA was isolated from dried or fresh fruiting bodies or mycelia. Some taxa proved extremely difficult to extract, particularly those with dark pigments (e.g., *Craterellus fallax*), and protocols were modified to reduce the concentra-

TABLE II. Taxa examined, with selected authors' familial placements

Taxon	Collection[a]	Herbarium	Donk (19)	Corner (12, 14)	Jülich (43)	GenBank nuc-ssu-rDNA	GenBank mt-ssu-rDNA
Cantharellus cibarius	DSH94-006	F	Cantharellaceae	Cantharellaceae	Cantharellaceae	AF184176	AF185966
Cantharellus lutescens	DAOM199243	DAOM	Cantharellaceae	Cantharellaceae	Cantharellaceae	AF184177	AF185967
Cantharellus sp.	B&C001	F	Cantharellaceae	Cantharellaceae	Cantharellaceae	AF184178	AF185968
Cantharellus tubaeformis	DSH93-209	F	Cantharellaceae	Cantharellaceae	Cantharellaceae	AF026636	AF026678
Clavaria acuta	HS3785	SFSU	Clavariaceae	Clavariaceae	Clavariaceae	AF184180	AF185969
Clavaria zollingeri	DED3622	SFSU	Clavariaceae	Clavariaceae	Clavariaceae	AF184181	AF185970
	Wiejek 41	WTU				AF184182	AF185971
Clavariadelphus ligulus	21648	F	Clavariaceae	Clavariadelphaceae	Clavariadelphaceae	AF184183	AF185972
Clavariadelphus pistillarus	37813	TENN	Clavariaceae	Clavariadelphaceae	Clavariadelphaceae	AF026639	AF026681
Clavariadelphus unicolor	36248	TENN	Clavariaceae	Clavariadelphaceae	Clavariadelphaceae	AF184185	AF185973
Clavulina cinerea	JFA10798	WTU	Clavulinaceae	Clavulinaceae	Clavulinaceae	AF184186	AF185974
Clavulina cristata	DAOM159321	DAOM	Clavulinaceae	Clavulinaceae	Clavulinaceae	AF026640	AF026682
Clavulina ornatipes	DED2869	SFSU	Clavulinaceae	Clavulinaceae	Clavulinaceae	AF184188	—
Clavulinopsis fusiformis	EP96-004	F	Clavariaceae	Clavariaceae	Clavariaceae	AF184189	AF185975
Craterellus cornucopioides	Danell43[b]		Cantharellaceae	Cantharellaceae	Craterellaceae	AF184190	AF185976
Craterellus fallax	DSH96-003	F	Cantharellaceae	Cantharellaceae	Cantharellaceae	AF184191	AF185977
Gerronema chrysophyllum	RHP42170	F			Tricholomataceae	AF184193	AF185978
Gloeocantharellus purpurascens	51702	TENN	Gomphaceae	Gomphaceae	Gomphaceae	AF184194	AF185979
Gomphus bonarii	REH1982	F	Gomphaceae	Gomphaceae	Gomphaceae	AF184195	AF185980
Gomphus floccosus	DSH94-002	F	Gomphaceae	Gomphaceae	Gomphaceae	AF026637	AF026679
Hydnum repandum	EP96-001	F	Hydnaceae		Hydnaceae	AF026641	AF026683
Hygrocybe conica	HDT48309	SFSU			Hygrophoraceae	AF184198	AF185981
Hygrophorus eburneus	HDT54064	SFSU			Hygrophoraceae	AF184199	AF185982
Lentaria byssiseda	*HDT5502	TENN	Gomphaceae	Ramariaceae	Lentariaceae	AF184200	AF185983
Macrotyphula cf. juncea	*DM-975	MIN.	Clavariaceae	Clavariadelphaceae	Clavariadelphaceae	AF184201	AF185984
Multiclavula mucida[c]	DSH96-058	F	Clavariaceae	Clavariaceae	Clavariaceae	AF026613	AF026659
Pterula aff. epiphylloides	DM-937	MIN.	Clavariaceae	Pterulaceae	Pterulaceae	AF184204	AF185985
Ramaria formosa	HS1788	TENN	Gomphaceae	Ramariaceae	Ramariaceae	AF184205	AF185986
Ramaria stricta	*HDT5474	TENN	Gomphaceae	Ramariaceae	Ramariaceae	AF026638	AF026680
Typhula phacorrhiza	DSH96-059	F	Clavariaceae	Clavariadelphaceae	Typhulaceae	AF026630	AF026686, AF026687

[a] Accessions are dried fruiting bodies, except those marked with *, which are mycelial cultures.
[b] Accession is a yellow mutant of the species (1, 64).
[c] Also known as *Stichoclavaria mucida*.

tions of these pigments. Fragments of fruiting bodies were first soaked in a buffer of 20% DMSO, 250 mM EDTA, and saturated NaCl (S. Rehner pers comm) 1–3 d, then rinsed with 1× TE pH 8.0 (10 mM Tris-HCl, 1 mM EDTA) 10 min. Extraction protocol was as follows: a small fragment (0.25 cm^3 or less) of fungal tissue was placed in a 1.5 mL microcentrifuge tube with 400 μL hot (60 C) 1% SDS extraction buffer and sterile sand. Tube contents were homogenized with a plastic pestle fitted into a hand drill (recalcitrant tissue was ground in a mortar under liquid nitrogen, then added to hot buffer). Tubes were incubated at 60 C 10–30 min, then extracted once with 25:24:1 phenol : chloroform : isoamyl alcohol and once with 24:1 chloroform : isoamyl alcohol. DNA was removed from solution using Geneclean II (Bio 101, La Jolla, California) and eluted into 50 μL 1× TE pH 8.0 (10 mM Tris-HCl, 1 mM EDTA). Serial dilutions of genomic DNA (1:10–1:1000) were used as template for the polymerase chain reaction.

Two unlinked genes were examined: mitochondrial small subunit rDNA (mt-ssu-rDNA) and nuclear small subunit rDNA (nuc-ssu-rDNA). Amplification and sequencing used the primers MS1 and MS2 (91) for mt-ssu-rDNA, and SR1c and NS41 (35) for nuc-ssu-rDNA. Double-stranded PCR products were purified using Geneclean II (Bio 101, La Jolla, California) or QIAquick spin columns (QIAGEN, Inc., Chatsworth, California). PCR and sequencing parameters were as described by Hibbett and Donoghue (36). Sequences were edited and assembled using SeqEd v. 3.0.1 (Applied Biosystems, Inc., Foster City, California) or Sequencher v. 3.0 (Gene Codes Corp., Ann Arbor, Michigan).

Sequences were aligned manually in SeqApp v. 1.9a169 and PAUP v. 3.1.1 (86); automated alignment algorithms were ineffective due to extensive length variation. For the number of nucleotides sequenced for each gene fragment, the size of the data matrix after introduction of alignment gaps, and the number of potentially phylogenetically informative characters included in analyses, see TABLE III. The mt-ssu-rDNA alignment was divided into seven sections following Hibbett and Donoghue (36): blocks 1, 3, 5, and 7 were aligned across all taxa, but blocks 2, 4, and 6 exhibited extreme variability and were excluded. Certain regions could not be aligned for divergent individuals and were scored as missing data for those taxa (mt block 1: 43 bp of both *Clavaria zollingeri* isolates; mt block 7: 53 bp of *Sparassis spathulata*; nuc: 237 bp of *Cantharellus cibarius* and 122 bp of remaining species of *Cantharellus* and *Craterellus*). One hundred and fifty-three bp of the nuc-ssu-rDNA were not comparable across all taxa but could be aligned within subsets; corresponding positions in the remaining taxa were scored as missing data. *Clavulina ornatipes* was not sequenced for the mt-ssu-rDNA and was scored as missing for all mt-rDNA positions in combined analyses. Alignments are deposited in TreeBASE.

Three data sets were developed to explore sensitivity of results to inclusion or exclusion of ambiguously aligned regions (see TABLE III). Dataset 1, the most inclusive, omitted only the beginnings and ends of sequences (124 bp), the unalignable mt-ssu-rDNA blocks 2, 4, and 6, and sites scored as missing for all but a single isolate. Dataset 2, the intermediate exclusion set, further excluded regions where the positioning of gaps was particularly ambiguous (128 bp). Dataset 3, the most exclusive set, additionally omitted an extremely variable region of mt block 5 (106 bp), and all characters that were scored as missing for more than 10% of the taxa.

Dataset 2, the intermediate exclusion set, was used to analyze sequences for the two genes separately. Analyses of the mitochondrial gene alone excluded *Clavulina ornatipes*. Analyses were performed on the combined data from both genes using all three datasets.

After two well-supported clades (designated "gomphoid-phalloid" and "stichic") were identified in analyses including all taxa, two new alignments were constructed that included only members of each clade. This reduced the total number of gaps required for alignment, and allowed inclusion of additional characters from regions that were too divergent to be aligned across the complete taxon set (see TABLE III).

Phylogenetic analyses were conducted using PAUP 3.1.1 (86) and test version 4.0d54 of PAUP* (written by David L. Swofford) on a Power Macintosh 8500/220 and Sun workstation. Heuristic searches were performed, with 100 random stepwise addition replicates with MULPARS on, steepest descent off, and TBR branch swapping. A two-step search was performed: first, no more than two trees were saved from each replicate, then exhaustive swapping was performed on all of the most parsimonious trees discovered. The resulting trees were rooted with *Tremella,* as suggested by the results of Swann and Taylor (85). One thousand bootstrap replicates were performed with the following settings: MULPARS option off, simple addition sequence, heuristic search, and TBR branch swapping. Analyses of the two subset alignments (gomphoid-phalloid and stichic) used the branch-and-bound search algorithm, which guarantees discovery of all most parsimonious trees.

RESULTS

The number of included, variable, and parsimony-informative characters for each data set is shown in TABLE III, along with the number and length of optimal trees found in each analysis. Independent analyses of mt-ssu-rDNA and nuc-ssu-rDNA suggest that there is evolutionary rate heterogeneity among lineages in both genes. In the mt-rDNA tree (FIG. 1), there are long branches leading to *Clavaria zollingeri* (33 steps), *Sparassis spathulata* (49 steps), the branch linking these three isolates (53 steps), and the branch linking these taxa to *Stichoclavaria* (34 steps). These are four of the five longest branches in the tree; the fifth consists of the 45 autapomorphic changes leading to *Boletus satanas*. In the nuc-rDNA tree (FIG. 2), 63 autapomorphic changes lead to *Cantharellus cibarius,* and there is a long branch of 38 steps supporting monophyly of Cantharellaceae. The next longest branch is 35 steps, leading to *Dacrymyces chrysospermus,* at the base of tree; no other branch is more than 25 steps long. The most obvious conflict

TABLE III. Description of the various data sets analyzed and the most parsimonious trees found

Data set	Size of Matrix	Characters			Shortest Trees	
		Included	Variable	Informative	Number	Length
Inclusive[a]	4383	1084	634	424	56	2629
Intermediate[a]	4383	956	522	346	4458	2022
Exclusive[a]	4383	744	430	283	64	1469
Mt-ssu-rDNA[a,b]	3303	311	242	183	372	1234
Nuc-ssu-rDNA[a]	1079	645	290	163	>4800	682
Gomphoid-phalloid[c]	2357	898	202	90	1	332
Stichic[d]	1783	1127	376	169	2	419

[a] Single alignment including all 65 taxa.
[b] *Clavulina ornatipes* was excluded.
[c] New alignment including only the 12 taxa in the gomphoid-phalloid clade.
[d] New alignment including only the 11 taxa in the stichic clade.

between the two gene phylogenies concerns relationships of taxa on these long branches. The mt-rDNA tree (FIG. 1) depicts monophyly of *Clavaria zollingeri* and *Sparassis spathulata* and places these taxa as the sister group of *Stichoclavaria,* although with less than 70% bootstrap support. The nuc-rDNA tree (FIG. 2), in which these taxa are not associated with unusually long branches, supports monophyly of all *Clavaria* species and *Clavulinopsis,* and places *Sparassis* as the sister group of *Laetiporus.* The mt-rDNA tree (FIG. 1) gives strong support (99% bootstrap) for the monophyly of Cantharellaceae and *Hydnum,* but the nuc-rDNA tree (FIG. 2) places *Cantharellus* and *Craterellus* near the base of the phylogeny, and leaves *Hydnum* with the remainder of the stichic clade. Other nodes that differ between the two gene trees either collapse in the strict consensus of equally parsimonious trees or receive less than 60% bootstrap support from one or both genes.

Results of analyses of the three exclusion sets of the combined data (datasets 1–3) differed slightly in bootstrap values and degree of resolution of the strict consensus tree, but no conflicting nodes received even moderate (>50%) bootstrap support. Because the major conclusions of this study are congruent with all three sets of analyses, only results of dataset 2 will be presented. Combined analyses (FIGS. 3, 4) place *Clavaria zollingeri* and *Sparassis* together and support monophyly of Cantharellaceae and *Hydnum,* reflecting the mt-rDNA results (FIG. 1). The branch leading to *Clavaria zollingeri* and *Sparassis* is the longest in the tree (68 steps). Furthermore, two of the three next longest branches lead to *Sparassis* itself (60 steps) and *Clavaria zollingeri* (63 steps). The remaining unusually long branch leads to the divergent *Cantharellus cibarius* (64 steps). The strict consensus tree (FIG. 4) does not resolve relationships of stichic taxa, but 71% of the bootstrap replicates support monophyly of stichic taxa. Lack of resolution in the strict consensus tree is due to conflicting placements of *Clavaria zollingeri* and *Sparassis;* alternate equally parsimonious positions are marked with dashed branches in FIGS. 3, 4. When *Clavaria zollingeri* and *Sparassis* were excluded from analyses, monophyly of stichic taxa was supported by all most parsimonious trees and 100% of bootstrap replicates.

Cantharelloid and clavarioid fungi appear in four groups (FIG. 3). *Gomphus, Ramaria, Gloeocantharellus, Lentaria,* and *Clavariadelphus* form a clade including *Pseudocolus, Geastrum,* and *Sphaerobolus* (henceforth referred to as the gomphoid-phalloid clade), with 100% bootstrap support. The stichic genera *Cantharellus, Craterellus, Hydnum, Clavulina,* and *Stichoclavaria* are monophyletic, including *Sparassis* and *Clavaria zollingeri* in some of the most parsimonious trees. *Clavicorona* is the sister group of *Auriscalpium* and *Lentinellus.* The remaining clavarioid fungi are nested within the clade including most of the gilled fungi and the polypore *Fistulina hepatica* (henceforth termed the euagaric clade after Hibbett et al 37).

Restricting attention to members of each of the first two clades (gomphoid-phalloid and stichic) allowed unambiguous alignment of more of the sequence data. Compared to the alignment including all 64 taxa, fewer gaps were required, reducing the matrix length, and reduced homoplasy provided fewer, shorter most parsimonious trees with better-resolved fine-scale relationships (TABLE III). The relationships supported by the single most parsimonious tree for the gomphoid-phalloid clade realignment (FIG. 5) are congruent with those supported by some of the most parsimonious trees for analyses including all taxa (e.g., FIG. 3). *Gomphus* is monophyletic (99% bootstrap), and closely related to *Ramaria formosa.* Although *Ramaria stricta* is the sister group of this

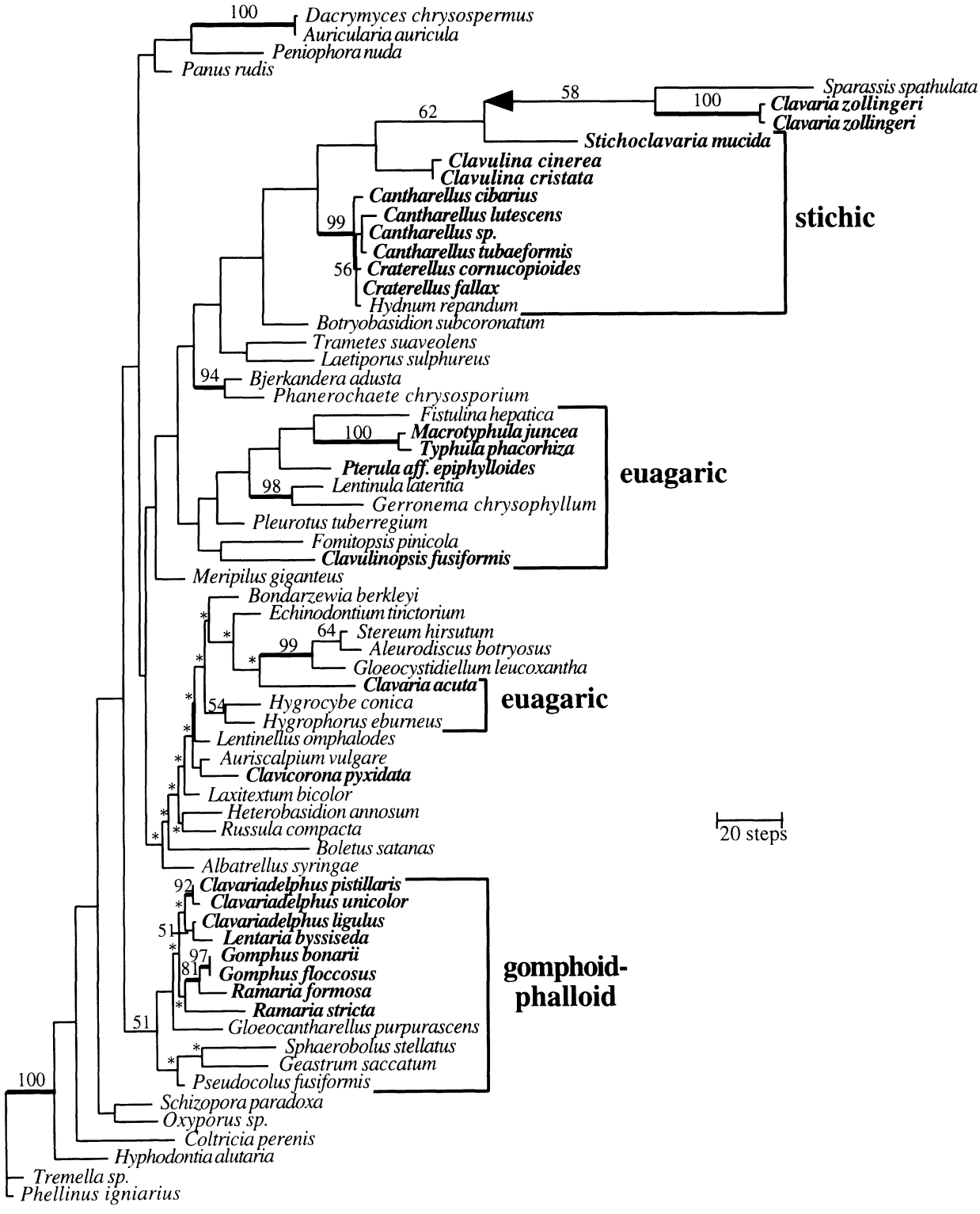

FIG. 1. Phylogram of mt-ssu rDNA gene tree. One of 372 equally parsimonious trees. L = 1234, CI = 0.332, RI eq 0.583, RC = 0.194. The following conventions are used in all figures: cantharelloid and clavarioid taxa are in boldface, bootstrap values (greater than 50% in FIGS. 1, 2, 4–6) are indicated next to the appropriate branch, branches receiving >70% bootstrap support are thickened, * indicates branches that collapse in the strict consensus of all most parsimonious trees.

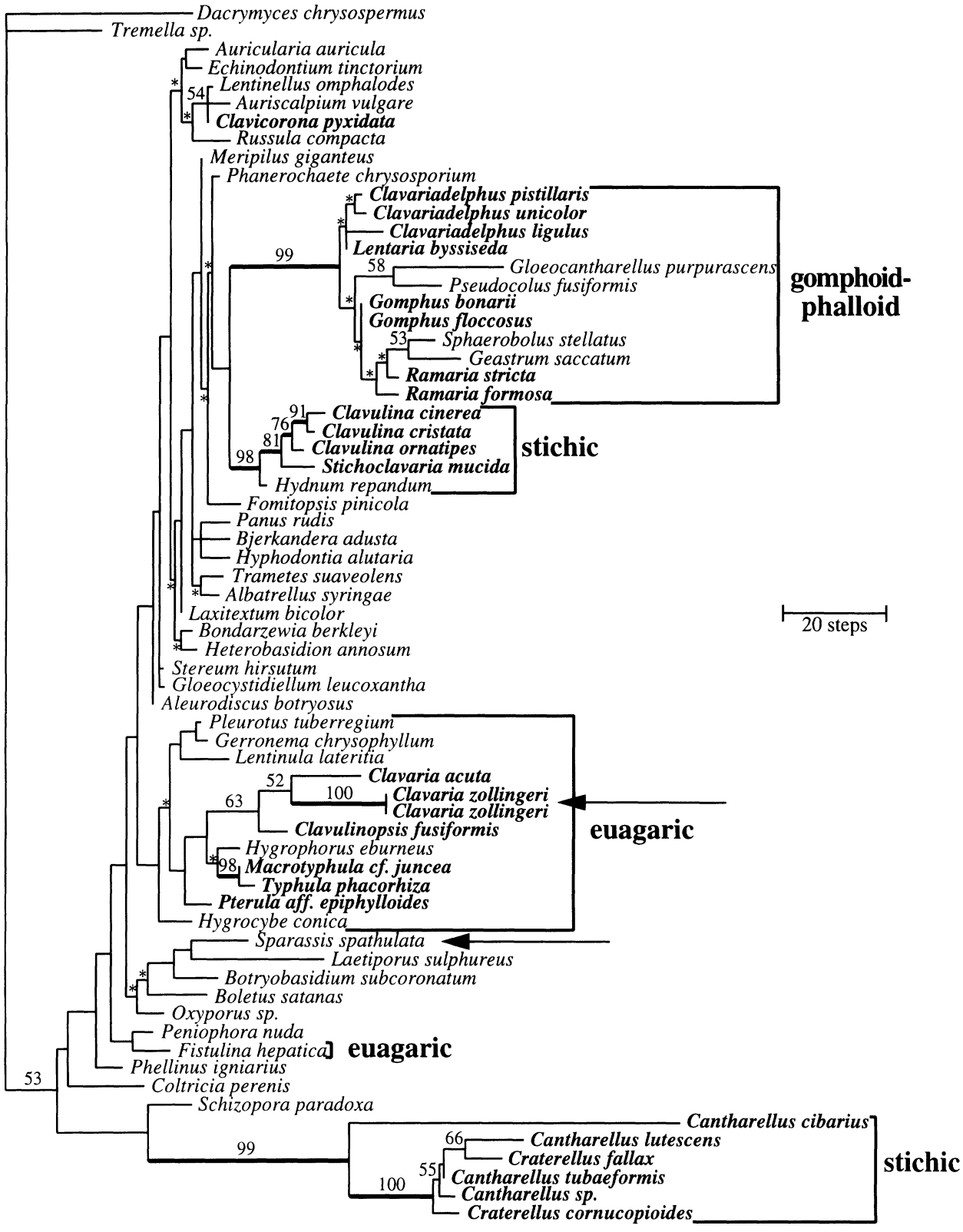

FIG. 2. Phylogram of nuc-ssu rDNA gene tree. One of >4800 equally parsimonious trees. L = 682, CI = 0.526, RI = 0.732, RC = 0.385. See FIG. 1 for explanation of conventions.

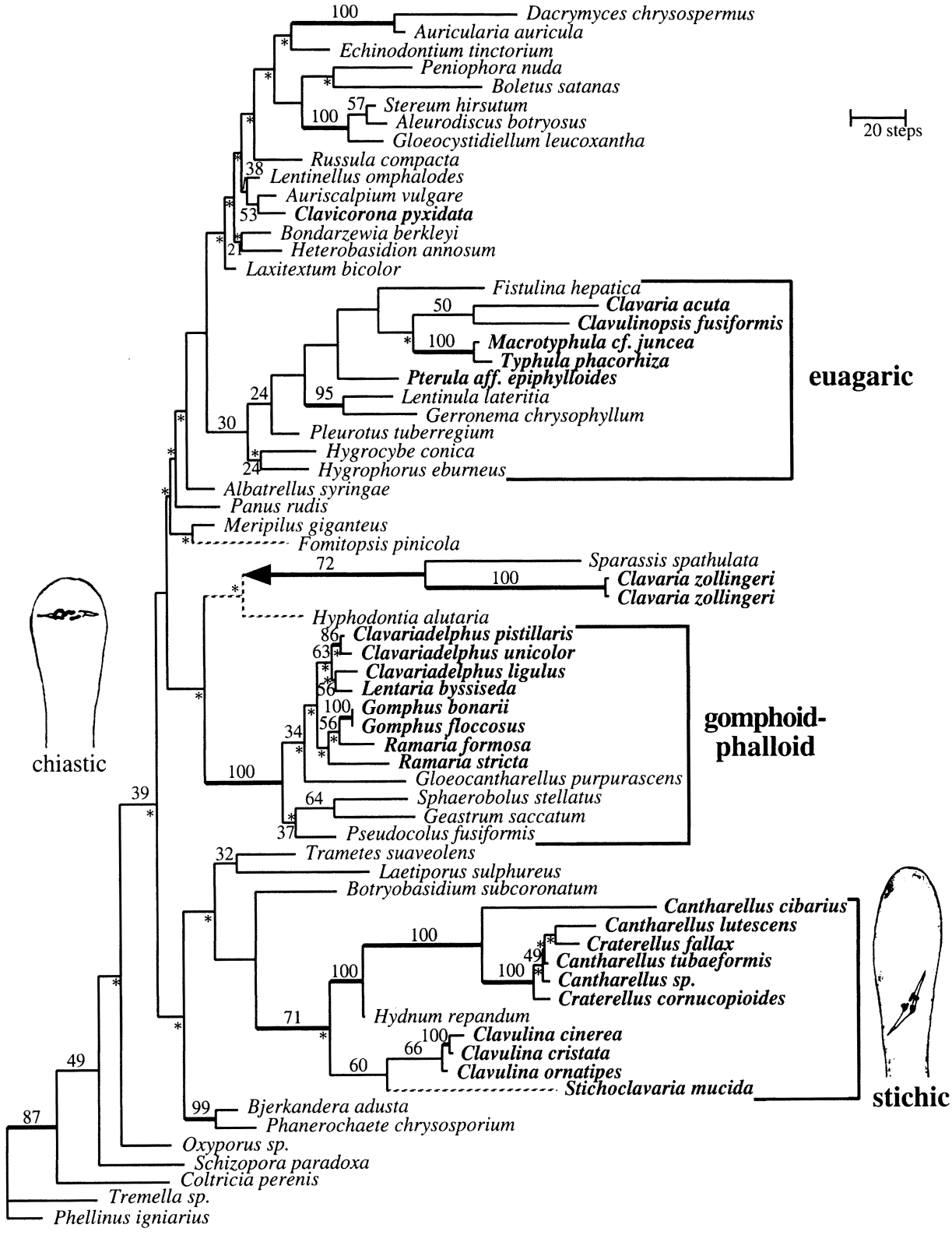

FIG. 3. Phylogram of combined data for mit-ssu rDNA and nuc-ssu rDNA. One of 4458 equally parsimonious trees, see Methods for analysis parameters. L (length) = 2022, CI (consistency index) = 0.380, RI (retention index) = 0.606, RC (rescaled consistency index) = 0.231. See FIG. 1 for explanation of conventions. In this figure, dashed branches indicate alternate placements of the branch leading to *Sparassis spathulata* and *Clavaria zollingeri* (arrow), which are reflected in FIGS. 1, 2. Line drawings (after 41) depict stichic vs chiastic meiotic division in immature basidia.

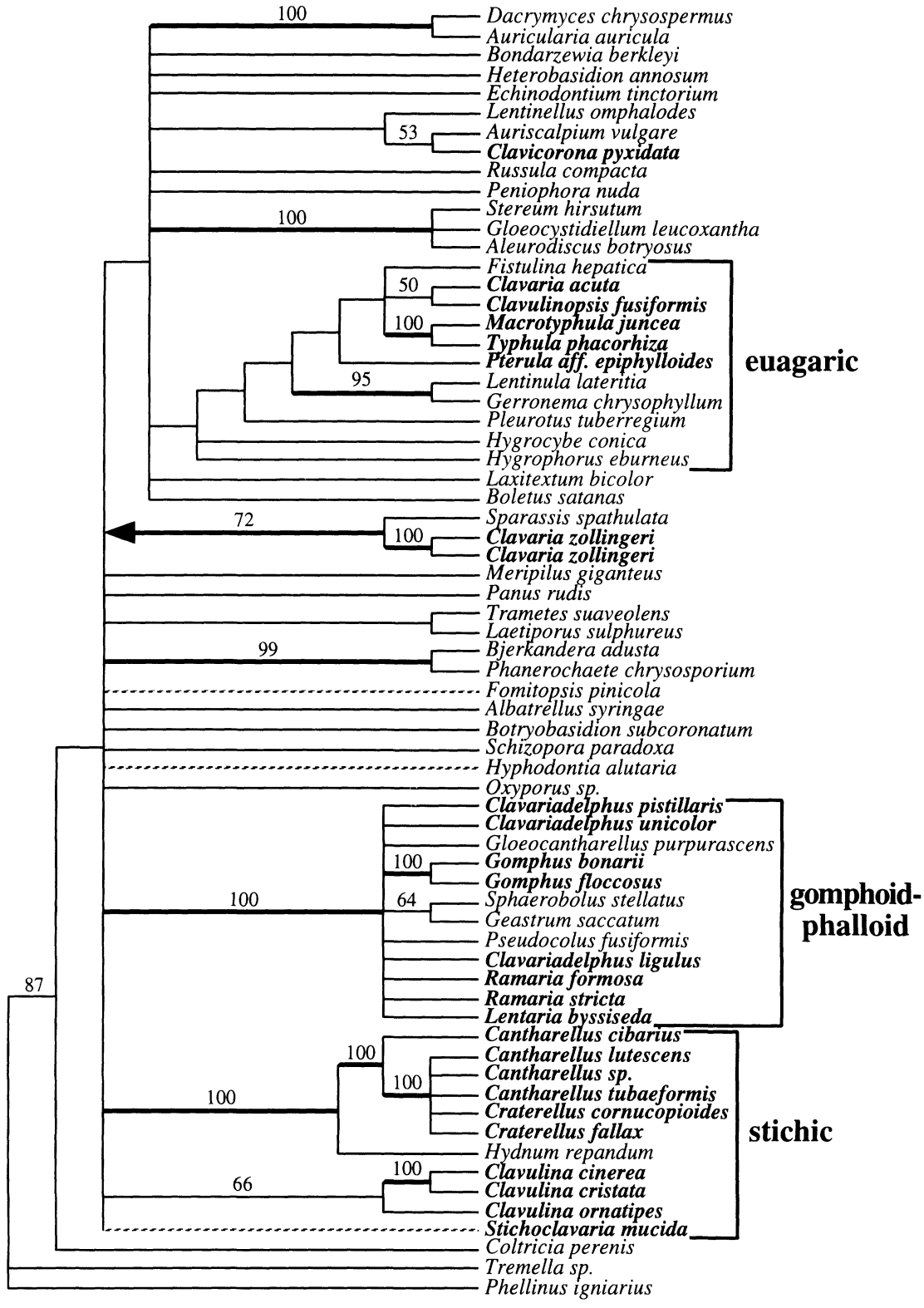

FIG. 4. Strict consensus tree for combined data. Consensus of 4458 equally parsimonious trees, L eq 2022, normalized CFI (consistency fork index) = 0.422. See FIG. 1 for explanation of conventions. Dashed branches indicate alternate placements of the branch leading to *Sparassis spathulata* and *Clavaria zollingeri* (arrow).

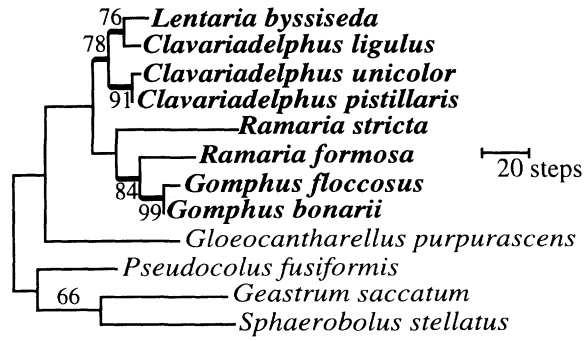

FIG. 5. Single most parsimonious phylogram for realigned combined data of gomphoid-phalloid clade. L = 332, CI = 0.782, RI = 0.557, RC = 0.406. See FIG. 1 for explanation of conventions used in figure. Topology is congruent with some of the most parsimonious trees for analyses including all taxa (e.g., FIG. 3). Rooted after FIG. 3 and Hibbett et al (37).

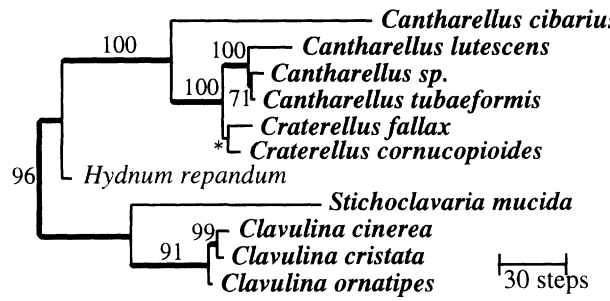

FIG. 6. One of the two most parsimonious trees for realigned combined data of stichic taxa. L = 419, CI = 0.924, RI = 0.921, RC = 0.850. See FIG. 1 for explanation of conventions. Topology is congruent with some of the most parsimonious trees for analyses including all taxa. Rooted after FIG. 3.

clade in the most parsimonious tree, bootstrapping does not support monophyly of *Gomphus* and *Ramaria*. The genus *Ramaria* appears to be paraphyletic. *Clavariadelphus pistillaris* and *C. unicolor* are sister taxa, as are *C. ligulus* and *Lentaria byssiseda*; *Clavariadelphus* is monophyletic if *L. byssiseda* is included. The *Clavariadelphus* lineage is nested within Gomphaceae, although bootstrap support is weak. *Gloeocantharellus* appears to be the basal lineage within Gomphaceae, but its position is not supported by bootstrapping. *Pseudocolus, Geastrum, and Sphaerobolus* are weakly supported as the monophyletic sister group of Gomphaceae.

Among stichic taxa, *Cantharellus lutescens* and *C. tubaeformis* form a clade which is the sister group of *Craterellus fallax* and *C. cornucopioides* (FIG. 6). There is strong support (100%) for the monophyly of these taxa, to the exclusion of *Cantharellus cibarius*. *Hydnum repandum* is the sister group of Cantharellaceae. Unfortunately, attempts to amplify DNA isolated from *Pseudocraterellus* were unsuccessful. *Clavulina* is monophyletic and is the sister group of *Stichoclavaria*.

DISCUSSION

In many cases, inclusion of additional characters in phylogenetic analysis increases the probability of correctly estimating the underlying tree topology (87). But inclusion of ambiguously-aligned regions introduces characters whose homology is questionable. Furthermore, inclusion of phylogenetically informative characters for which multiple taxa are scored as missing sometimes can result in spurious resolution of artificial clades during parsimony analysis (53). Thus, there is a dilemma in phylogeny reconstruction of omitting large numbers of characters vs including characters that may add noise or be positively misinformative. However, results from analyses of the three exclusions sets suggest that these factors did not affect conclusions of this study, since all phylogenetic resolution receiving even moderate bootstrap support from any of the three exclusion sets tested is compatible with results from all three.

Possible causes for incongruence of the underlying phylogeny of two genes from the same taxa include: incomplete lineage sorting, hybridization, and other modes of horizontal transfer (54). The first two phenomena most likely occur among closely related species; this study focuses on relationships among genera and families that presumably diverged long ago. Horizontal transfer of the genomic regions used in this study has never been reported. Thus, we expected the mt-ssu-rDNA and nuc-ssu-rDNA sequences of the taxa in this study to represent the same underlying phylogeny. This expectation was supported by comparison of bootstrap values for the two gene phylogenies (FIGS. 1, 2)—all positive conflict between the two trees receives less than 70% bootstrap support in at least one gene phylogeny.

The most distinctive topological conflict between the two gene trees concerns the placement of *Clavaria zollingeri* and *Sparassis spathulata*. Mitochondrial data depict these taxa as monophyletic, and place them within a clade that is otherwise stichic (FIG. 1). This differs from their placement based on nuc-ssu-rDNA (FIG. 2) or morphological characters, but strong support for any of these placements is lacking. Previous analyses of 1.2 kb of nuc-ssu-rDNA and the MS1/MS2 fragment of mt-ssu-rDNA support monophyly of *Sparassis, Phaeolus schweinitzii,* and *Laetiporus sulphureus,* but only with moderate (50%) bootstrap support (35). However, when additional taxa are sampled and complete nuc-ssu-rDNA se-

quences (1.8 kb) are included, bootstrap support for this clade rises to 98% (37). *Sparassis, Laetiporus,* and *Phaeolus* all have ellipsoid-ovoid, smooth, inamyloid spores, produce a brown rot, and can cause root and butt rot of living trees, although host ranges differ. Taken together, the ecological and anatomical characters and nuc-ssu-rDNA evidence suggest that the correct placement of *Sparassis* is with *Laetiporus.* The lengths of the branches leading to *Clavaria zollingeri, Sparassis,* and their putative sister taxa (see Results) in both the combined tree (FIG. 3) and the mt-rDNA gene tree (FIG. 1) suggest that long branch attraction could be responsible for their placement. In certain cases of grossly unequal branch lengths, parsimony analysis has been demonstrated to artificially connect extremely long branches that are unrelated in the true underlying phylogeny (27, 40). The branch leading to *Stichoclavaria* is the longest in the mt-ssu-rDNA tree (FIG. 1) if *C. zollingeri* and *Sparassis* are deleted (55 steps), suggesting that it is a likely candidate for this analytical artifact. Furthermore, monophyly of stichic taxa, *C. zollingeri,* and *Sparassis* receives only marginal (62%) bootstrap support, while monophyly of stichic taxa receives 92% bootstrap support in analyses of mt-ssu-rDNA that exclude the problematic *C. zollingeri* and *Sparassis.* Thus the mitochondrial data alone do not unambiguously support the placement of *C. zollingeri* and *Sparassis,* and furthermore, underlying phylogenetic signal supports monophyly of stichic taxa. Although branch lengths are more evenly distributed in combined analyses (FIG. 3), *Sparassis* and *C. zollingeri* are still extremely divergent and are grouped together. There is no support for their placement in the tree, resulting in three alternative placements (FIGS. 3, 4) and lack of resolution in the strict consensus of equally parsimonious trees (FIG. 4). In the most conservative estimate, data presented in this study are insufficient to resolve relationships of *Clavaria zollingeri* and *Sparassis.* However, if evidence from anatomical and ecological characters and nuc-ssu-rDNA are given precedence over the dubious mt-rDNA results, *Clavaria zollingeri* belongs with *Clavaria acuta* and *Clavulinopsis, Sparassis* is the sister group of *Laetiporus,* and neither are nested within the stichic clade.

A similar argument can be used to explain the polyphyly of stichic taxa found in the nuc-ssu-rDNA analyses (FIG. 2). The longest branches in the tree are found within the Cantharellaceae, and the only other branch that is nearly as long is at the base, leading to *Dacrymyces.* The rest of the stichic clade exhibits extremely short branches. We conclude that Cantharellaceae is probably drawn to a basal position in analyses based on nuc-ssu-rDNA because of its high degree of divergence. Its position as the sister group of *Hydnum repandum* receives unequivocol support in analyses with more evenly distributed branch lengths (FIGS. 1, 3).

Overview.—Higher level relationships of Homobasidiomycetes are not resolved by these analyses (FIG. 4). Nevertheless, in no analyses do cantharelloid and clavarioid fungi appear to form a basal, paraphyletic group from which the rest of the Hymenomycetes have been derived. Thus, this study provides no support for Corner's *Clavaria* theory of Homobasidiomycete evolution. Additionally, there is no evidence for a relationship between Cantharellaceae and the agarics *Gerronema* or *Hygrocybe,* supporting Donk's (19) and Heinemann's (33) conclusions that similarities of these genera to Cantharellaceae are due to convergence. Instead, it appears that many clavarioid fungi, traditionally placed in Clavariaceae, are derived from a lineage (designated euagaric) that also gave rise to the gilled mushrooms *Lentinula, Pleurotus, Hygrophorus, Hygrocybe,* and *Gerronema,* and to the polypore *Fistulina.*

Coral- and club-shaped fungi have been derived in four lineages, two of which have also given rise to cantharelloid fungi. The fruiting bodies of the nearest extant relatives of the different lineages represent a wide range of forms: gilled mushrooms, toothed fungi, puffballs, stinkhorns, and the cannon-ball fungus. Similar rapid evolution of fruiting-body macromorphology has been documented in diverse lineages of Homobasidiomycetes (7, 37, 38, 47, 59). The agarics *Neolentinus lepideus* and *Lentinellus* can produce clavarioid fruiting bodies under appropriate environmental conditions (9, 57, 61 p 184), and Donk (19 p 207–208) discusses several taxa whose fruiting bodies can be either corticioid or clavarioid. It appears that superficial similarity of form is not a good predictor of evolutionary proximity in the cantharelloid and clavarioid fungi. Instead, our results suggest that certain anatomical features are conserved within lineages that are otherwise morphologically diverse. For example, the coralloid *Clavicorona,* the toothed *Auriscalpium,* and the gilled *Lentinellus* form a monophyletic group characterized by amyloid spore ornamentation. One of the goals of this study was to evaluate putative synapomorphies for other lineages with cantharelloid and clavarioid members.

Gomphoid-phalloid clade.—*Gomphus, Ramaria,* and *Gloeocantharellus* are united by cyanophilic, warty spore ornamentation and by green reactivity to iron salts (TABLE I). *Gloeocantharellus* has a cantharelloid aspect, but has true gills and contains abundant gloeoplerous hyphae. Our results strongly support the accepted placement of *Gloeocantharellus* with *Gomphus* and *Ramaria,* indicating that *Gloeocantha-*

rellus represents an independent derivation of gills within the gomphoid-phalloid lineage, and is unrelated to any other agaric or boletoid fungi examined thus far.

Other fungi reported with spores and macrochemical reactivity similar to Gomphaceae include hydnoid *Beenakia* (44, 52, 60), and the resupinates *Kavinia,* which is toothed, and *Ramaricium,* which has a smooth hymenophore (19, 24, 52). Although these taxa are not represented in this study, sequences from the mitochondrial large subunit rDNA support placement of *Kavinia* with *Gomphus* and *Ramaria* (8), suggesting that spore morphology and iron salt reactivity may be a synapomorphy of this group. Another relative of Gomphaceae seems to be *Gautieria,* a false-truffle with striate, brown-pigmented spores that are also reported to be cyanophilous (46). Sequence data from mitochondrial large subunit rDNA (8) and nuclear large subunit rDNA (J. Spatafora pers comm) support placement of *Gautieria* with Gomphaceae. Petersen (71 p 15) reported that "the staining reaction and general construction of the spore wall" of *Gymnopilus* and the boletoid taxa, *Porphyrellus subflavidus, Strobilomyces confusus,* and *S. floccopus* are very similar to that of *Gomphus,* but relationships among these taxa and Gomphaceae have not been examined further.

Members of the club-shaped genus *Clavariadelphus* react green on contact with iron salts (TABLE I), reflecting the presence of pistillarin (56). These analyses provide 100% bootstrap support for the placement of *Clavariadelphus* within Gomphaceae, rejecting a relationship with Cantharellaceae and with Clavariaceae. Although *Clavariadelphus* spores are smooth, hyaline, and unreactive, like those of Cantharellaceae and Clavariaceae, this state appears to be plesiomorphic conservation of ancestral features.

Lentaria in the restricted sense is a homogenous group of branched, lignicolous clavarioid fungi characterized by white, smooth spores and thick-walled generative hyphae that give the fruiting body a leathery texture (67, 72). Corner (10, 14) included in the genus phycophilous, stichic species that Petersen (67) segregated into *Multiclavula* (= *Stichoclavaria*). Although Corner (10 p 24) left *Lentaria* in Clavariaceae, he noted a resemblance between *L. byssiseda* and the Stricta group of *Ramaria*; shared green reactions with iron salts and thick-walled skeletal hyphae led Petersen (65, 72 Fig. 10) to conclude that *Lentaria* s. s. was derived within *Ramaria.* Corner (14) moved *Lentaria* from Clavariaceae into a new family, Ramariaceae. Our results support Petersen's separation of *Multiclavula* (= *Stichoclavaria*) and *Lentaria* s. s., as well as the placement of the latter genus in Gomphaceae (FIG. 3), although *Lentaria byssiseda* appears to be nested within *Clavariadelphus* rather than *Ramaria* (FIG. 5).

Several other taxa have been reported to stain green on contact with iron salts. Petersen (68, 72) described green or gray-green reactions of *Cantharellus cibarius* and some species of *Clavulinopsis* (which he redefined as *Ramariopsis* in 1978), but later (78) reported that pistillarin, the compound responsible for the green reaction in Gomphaceae and *Clavariadelphus,* was not present in *Clavulinopsis.* Our results suggest that neither *Cantharellus* nor *Clavulinopsis* are related to Gomphaceae, indicating that green iron salt reactions in the absence of pistillarin are not phylogenetically informative. Welden (90) suggested that *Stereum radicans* (= *Stereopsis* Reid) was related to Gomphaceae and *Clavariadelphus,* since it also stains green on contact with iron salts, but its pistillarin content has not been examined and it was not represented in this study.

The remaining taxa in the gomphoid-phalloid clade are *Pseudocolus, Geastrum,* and *Sphaerobolus.* Bootstrap support for the placement of these gasteromycetes with Gomphaceae is unequivocal (100%), and inclusion of the rest of the nuclear 18S rDNA and additional taxa does not alter this result (37). Furthermore, sequences from nuclear large subunit rDNA (28S) support a relationship between Gomphales and Phallales (J. Spatafora pers comm). Relationships among stinkhorns, earth-stars, the cannon-ball fungus, and cantharelloid and clavarioid fungi have never been proposed in the taxonomic literature, and no morphological synapomorphy has yet been identified for this diverse clade. Although Pellegrini and Patrignani's (62) examination of septal pore apparatuses let them to suggest that "the genus *Clavariadelphus* could be placed closer to *Phallales* owing to the perforate parenthesome with small irregular holes," they observed intact dolipore septa in all *Ramaria* species examined. Fungi in the gomphoid-phalloid clade are remarkably ecologically and morphologically diverse, and have traditionally been examined by different groups of mycologists. Comparative studies of the anatomy and biochemistry of these taxa might elucidate morphological features that unite the lineage, and should be pursued. For example, iron salt reactions and pistillarin content of *Pseudocolus, Sphaerobolus,* and *Geastrum* should be investigated.

Stichic clade.—Although monophyly of stichic taxa is not supported by all analyses, placement of *Sparassis* and *Clavaria zollingeri* in the midst of an otherwise stichic clade is difficult to accept. Because such a relationship is contradicted by all evidence except mt-ssu-rDNA sequences, which may be susceptible to

long branch attraction of these taxa and do not provide strong bootstrap support, we reject the mt-ssu-rDNA results for *Sparassis* and *C. zollingeri* in favor of the placements supported by nuc-ssu-rDNA. Similarly, the removal of Cantharellaceae from the stichic clade to a basal position in the tree, seen only in the nuc-rDNA analyses (FIG. 2), can be explained by long branch attraction. Thus we conclude that stichic taxa form a monophyletic group, *Sparassis* and *Laetiporus,* both brown rot fungi, are sister taxa, and the genus *Clavaria* is most likely monophyletic and nested within the euagaric clade.

Our results provide strong (100% bootstrap) support for the monophyly of Cantharellaceae, but *Cantharellus* as previously defined appears to be paraphyletic (FIG. 6). *Cantharellus cibarius* is the sister taxon of a clade consisting of *C. tubaeformis, C. lutescens* (= *xanthopus,* see 20), *Craterellus fallax,* and *Cr. cornucopioides.* These results confirm earlier findings by Feibelman et al (26) and Dahlman et al (pers comm). Feibelman et al (26) recently proposed a new circumscription of genera within Cantharellaceae based on results from phylogenetic analyses of nuclear large subunit (28S) rDNA sequences. Feibelman et al included only three of the species in our study, but their conclusion that a clade containing *C. cibarius* can be separated from a clade including *Cr. fallax* and *C. tubaeformis* is concordant with our results (FIG. 6). They revised *Cantharellus* to contain *C. cibarius* and its relatives, and suggested that the genus *Craterellus* be expanded to include *C. tubaeformis* and *Pseudocraterellus sinuosus,* in addition to traditional members of *Craterellus* (e.g., *Cr. fallax, Cr. odoratus*). If our results are fitted to their generic circumscription, *C. lutescens* must also be transferred to *Craterellus.* Feibelman et al also evaluated some of the morphological features discussed in the Introduction, and concluded that "shape and texture seem to be more important [characters] than clamps, secondary septa, development, or hymenial configuration" in evaluating relationships of Cantharellaceae.

Analyses of carotenoid pigments of Cantharellaceae provide some support for the circumscription suggested by Feibelman et al (26). *Cantharellus lutescens, C. tubaeformis,* and other members of *Cantharellus* subg. *Phaeocantharellus* sensu Corner (12) accumulate carotenoids with aliphatic structure exclusively, while *C. cibarius* and other members of Corner's subgenus *Cantharellus* (roughly corresponding to genus *Cantharellus* sensu 26) accumulate predominantly bicyclic carotenoids (2, 28). However, published reports of pigment analyses provide conflicting results in some cases. For example, Arpin and Fiasson (2 p 84) state that "*C[r]. cornucopioides* links closely to the group *C. lutescens-C. tubaeformis,* from which it differs only in having a weaker carotenogenesis, with correspondingly relatively strong development of dark pigments of another sort." In contrast, Fiasson et al (30) found that *Cr. cornucopioides* was totally devoid of carotenoids, while *Cr. fallax,* which is otherwise very similar to *Cr. cornucopioides,* possessed the same carotenoids as *C. cibarius.* It is intriguing that carotenoid pigment structure seems to correlate with relationships supported by other characters, but until more taxa are examined and conflicting reports are resolved it is impossible to determine the pattern of pigment evolution within Cantharellaceae. Our data do support multiple derivation of bicyclic carotenoids in diverse lineages, since neither *Gerronema* nor *Clavulinopsis* are closely related to any members of Cantharellaceae.

Hydnum repandum is the sister group of Cantharellaceae, supporting Donk's (17, 19) and Petersen's (72) conclusions based on morphological similarity. Note that we are using Donk's (19) restricted definition of *Hydnum,* typified by *H. repandum;* the name *Dentinum,* which has been used for this group, is invalid (76). Although nuc-ssu-rDNA data taken alone remove Cantharellaceae from the stichic clade (FIG. 2), the extreme divergence of the nuclear rDNA of Cantharellaceae (18S: note the long branches in FIG. 2; ITS: ref. 25; 28S: J. Spatafora pers comm) makes it very likely that long branch attraction is responsible for the placement of Cantharellaceae near the base of the tree and for the absence of support from bootstrapping when the nuc-ssu-rDNA is taken alone. The branch length disparity of Cantharellaceae is much less severe in combined analyses, which provide unequivocal (100% bootstrap) support for the monophyly of Cantharellaceae and *Hydnum* (FIG. 3). Although *Hydnum* has a toothed hymenophore, it is similar to *Cantharellus* in color, aspect, anatomy, and flavor (TABLE I), and also has stichic nuclear division (80).

Stichic nuclear division (see FIG. 3) was first described by Juel (41), soon after which Maire (55) proposed a classification scheme for the fleshy basidiomycetes based on the distinction between stichic and chiastic basidia. Ulbrich (88) erected new genera and families for stichic taxa, but his classification was largely ignored by subsequent literature. Many authors since have criticized the use of this character for taxonomy (10 p 27, 12 p 11, 43, 66, 83). Although Donk's early work (17) gave strong weight to stichic nuclear division, placing *Clavulina* and *Hydnum* s. s. within Cantharellaceae, he later (18, 19) revised his opinion, removing *Clavulina* to its own family on the grounds that it was so evolutionarily divergent that its nearest relatives could not be determined. Our results support Maire's (55), Ulbrich's (88), and Donk's

original (17) concepts of close relationships among all stichic taxa.

Petersen (67) segregated small, lichenized, unbranched clavarioid fungi into the genus *Multiclavula,* but suggested that *Multiclavula* belonged in a generic complex with *Clavaria* (72). Hubbard and Petersen (39) concluded that Juel (42) was likely examining a *Multiclavula* when he described the nuclear state of *Clavaria falcata.* In 1928, Ulbrich erected the family Stichoclavariaceae, including two genera—*Stichoclavaria,* typified by *C. falcata,* and *Stichoramaria,* including *S. rugosa, S. cristata, S. cineria,* and *S. grisea*—for stichic clavarioid fungi. Although Ulbrich's *Stichoramaria* is a synonym for the older *Clavulina,* we concur with Hubbard and Petersen's suggestion that "*Stichoclavaria* should be reconsidered as the correct name for the *Multiclavula* complex." Our results support Petersen's segregation of *Stichoclavaria* from other clavarioid fungi, but suggest that similarities between *Stichoclavaria* and *Clavaria* are due to convergence; the nearest relatives of *Stichoclavaria* are taxa with the same mode of meiotic nuclear division.

The only reportedly stichic genera not represented in this study are the resupinate fungi *Clavulicium* and *Sistotrema. Clavulicium* is anatomically very similar to *Clavulina* (5), while *Sistotrema* possesses unique uniform basidia that make its relationship to other basidiomycetes difficult to ascertain; no known data contradict a relationship with the stichic clade revealed by our analyses. Because such a wide range of chiastic genera were sampled, it is likely that stichobasidia are indeed uniquely derived and have never been reversed. Still, nuclear behavior during meiosis has yet to be examined in many groups of basidiomycetes. Attempts to identify correlated characters, such as narrow, elongate basidia, have been strongly criticized (19 p 220). For example, *Hygrocybe* is anatomically very similar to stichic fungi, notably in basidial shape (34), but is reported to be chiastic (55). If stichobasidia are as phylogenetically informative as these results suggest, examination of more taxa may identify other relatives of Cantharellaceae, *Hydnum, Stichoclavaria,* and *Clavulina.*

Euagaric clade.—Although the evolutionary relationships of the remaining clavarioid genera are not definitively resolved by these data, they appear to be nested within the lineage containing the major radiation of gilled mushrooms (FIGS. 3, 4). The monophyly of *Macrotyphula juncea* and *Typhula phacorrhiza* is well supported (100% bootstrap), suggesting that earlier placements of *Macrotyphula* with *Clavariadelphus* (14, 43) were erroneous. *Clavaria acuta* and *Clavulinopsis* (= *Ramariopsis*) *fusiformis* are monophyletic in most analyses, although without strong bootstrap support. A clade including these clavarioid fungi and *Pterula,* the mushrooms *Hygrocybe, Hygrophorus, Pleurotus, Gerronema, Lentinula,* and the polypore *Fistulina* appears in all of the most parsimonious trees from the combined data (FIG. 4). Although this clade does not receive strong bootstrap support (30%), analyses including more non-cantharelloid or clavarioid taxa and the rest of the nuc-ssu-rDNA provide 97% bootstrap support for the placement of *Typhula* and *Fistulina* with *Pleurotus, Lentinula,* and other members of the euagaric clade (37). Future mycological studies cannot assume that mushrooms and coral and club fungi represent distinct lineages. Furthermore, it is now clear that coral and club fungi have been derived multiple times from diverse lineages, and do not represent an ancestral group that gave rise to the more complex fruiting forms found in the Basidiomycetes.

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