

MULTIPLE ORIGINS OF SEQUESTRATE FUNGI RELATED TO *CORTINARIUS* (CORTINARIACEAE)¹

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The aim of the present study was to investigate the phylogeny and evolution of sequestrate fungi (with gastroid or partially exposed basidiomes) in relation to their gilled relatives from the Cortinariaceae (Basidiomycetes). Phylogenetic analyses of 151 ITS sequences from 77 gilled species and 37 sequestrate taxa were performed using maximum parsimony and maximum likelihood methods. Results show that sequestrate basidiome forms occur in all three major ectomycorrhizal lineages of Cortinariaceae: the clades *Cortinarius*, *Hebeloma/Hymenogaster/Naucoria*, and *Descolea*. However, these forms do not appear within the saprobic outgroup *Gymnopilus*, indicating multiple origins of sequestrate forms from ectomycorrhizal ancestors. Additionally, within the *Cortinarius* clade sequestrate forms have multiple origins: emergent *Cortinarius* spp., *Thaxterogaster*, *Quadrifora*, *Protoglossum*, and two *Hymenogaster* spp. (*H. remyi*, *H. subilacinius*) share common ancestors with *Cortinarius* spp., but these sequestrate genera are not closely related to each other (with exception of *Thaxterogaster* and *Quadrifora*). *Hymenogaster* sensu stricto, *Setchelliogaster*, and *Descomyces* were placed in the two other major clades. Thus, sequestrate taxa evolved independently many times within brown-spored Agaricales. Furthermore, emergent, secotioid, and gastroid forms have evolved independently from each other, and so are not necessarily intermediate forms. After their establishment, these apparently morphologically stable taxa show a tendency to radiate.

Key words: *Cortinarius*; *Descomyces*; *Descolea*; *Hymenogaster*; phylogeny; *Protoglossum*; *Thaxterogaster*; *Quadrifora*.

The term sequestrate has been applied to basidiomycetes or ascomycetes with spores that are not forcibly discharged (statismospores) and whose basidia or asci mature inside enclosed, mostly hypogeous (below ground) fruit bodies or inside only partially exposed (emergent) fruit bodies (Bougher and Lebel, 2001) (Figs. 1–3). Sequestrate ascomycetes have commonly been called true-truffles, but for sequestrate basidiomycetes many different terms describing a particular fruit body habit have been used: trufflelike, false truffles, gastroid (a peridium encloses a loculate or highly convoluted gleba lacking a stipitate columella), and secotioid (the margin of the pileus remains appressed to the stipe and the lamellae are convoluted and anastomosed). The term sequestrate was proposed by Kendrick (1992) as a neutral term as regards phylogenetic relationship. Hence, sequestrate fungi encompass a polyphyletic group of diverse taxa often resembling particular fungi that forcibly eject their spores (Thiers, 1984).

In the past, the majority of sequestrate basidiomycetes have traditionally been placed in the Gasteromycetes because of their statismospory and enclosure of fertile tissue. However, similarities of certain gastroid and secotioid fungi with gilled fungi were soon noted, and various natural series were pro-

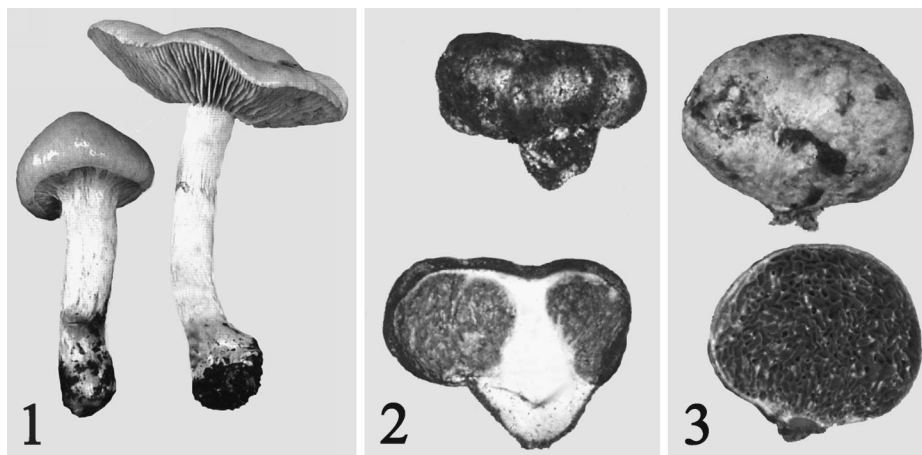
posed to show these relationships and possible directions of evolution (Smith and Singer, 1959; Heim, 1971). Such relationships were proposed also for *Cortinarius* (Pers.) S.F. Gray, perhaps the largest genus of agaricoid basidiomycetes. This genus of gilled fungi is recognized by the rusty brown basidiospores, which have roughened walls, the presence of a typically thin, cobweb-like partial veil (cortina), and a mutualistic (ectomycorrhizal) lifestyle. The tendency toward establishing a trufflelike habit from lamellate ancestors within the *Cortinarius* series was evidenced by gradually divergent morphologies. Three different sequestrate forms were thought to have been derived from *Cortinarius*: (1) strongly velate and partially hypogeous (emergent) *Cortinarius* taxa (Thiers and Smith, 1969); (2) secotioid *Thaxterogaster* spp. (Singer and Smith, 1963; Singer, 1951), and (3) gastroid *Hymenogaster* spp. (Thiers, 1984) (Figs. 1–3).

However, morphological studies alone have been inadequate to resolve the question of the origin and evolution of sequestrate taxa related to *Cortinarius*. Even in cases where affinities have been generally agreed upon, e.g., the relationship between *Cortinarius* and *Thaxterogaster*, the direction of evolution has long been debated (Smith, 1971; Singer, 1975; Thiers, 1984). Meanwhile, molecular studies have provided compelling evidence that taxa formerly classified in the Gasteromycetes have evolved repeatedly from nonsequestrate ancestors within the Homobasidiomycetes (Hibbett et al., 1997; Hibbett and Thorne, 2000). Most molecular studies have focused on Russulales or Boletales and their sequestrate counterparts: within Russulales, *Macowanites*, *Gymnomyces*, *Cystangium*, *Zelleromyces*, and *Arcangeliella* have arisen several times from *Russula* and *Lactarius* (Miller et al., 2001); within Boletales, the secotioid genus *Gastrovillus* is derived several

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Figs. 1–3. Basidiome types occurring in the *Cortinarius* clade. 1. Agaricoid basidiomes of *Cortinarius* sp. 2–3. Sequestrate basidiome types. 2. Secotioid basidiome type of *Thaxterogaster* sp. 3. Gastroid basidiome type of *Protoglossum* sp. Photo by Neale L. Bougher.

times from *Suillus* and is thus polyphyletic (Kretzer and Bruns, 1997), while the truffle-like *Rhizopogon* is monophyletic, sharing a common ancestor with *Suillus* (Johannesson and Martin, 1999; Grubisha et al., 2001). Surprisingly little molecular research has been focused on the linkages between the Agaricales (true gilled mushrooms) and their sequestrate relatives: only the relationship between the gilled mushroom *Coprinus* and its sequestrate relatives *Podaxis* and *Montagnea* (Hopple and Vilgalys, 1994, 1999), the relationship of the sequestrate *Weraroa* with *Strophariaceae* (Binder, Besl, and Bresinsky, 1997), and the relationship between the secotioid *Setchelliogaster* and the agaric *Descolea* have been demonstrated (Martin, Hogberg, and Llistosella, 1999; Martin and Roccabruna, 1999).

In this study we used ITS sequences to investigate the phylogeny of sequestrate taxa related to *Cortinarius*. Is there a link between *Cortinarius*, *Thaxterogaster*, and *Hymenogaster*? Do these sequestrate genera represent monophyletic groups? Is it appropriate to split *Hymenogaster* into morphologically more homogenous units (*Hymenogaster sensu stricto*, *Descomyces*, *Protoglossum*, *Quadriflora*, and *Timgrovea*) (Bougher and Castellano, 1993)? If monophyletic entities exist among these sequestrate forms, are morphological characters such as spore and peridium morphology useful for their delimitation?

MATERIALS AND METHODS

Specimens—For 77 taxa of Cortinariaceae (*Cortinarius*, *Rozites*, *Cuphocybe*, *Hebeloma*, *Naucoria*, *Descolea*, and *Gymnopilus*) and 37 sequestrate taxa (*Thaxterogaster*, *Protoglossum*, *Quadriflora*, *Hymenogaster*, *Descomyces*, and *Setchelliogaster*), 106 ITS sequences were generated, and 45 sequences were retrieved from GenBank. Collection data and GenBank accession numbers can be found at the AJB Supplementary Data web site (<http://ajbsupp.botany.org/>). For authors for fungal species names refer to Funindex (<http://194.131.255.3/cabipages/Names/NAMES.ASP>).

DNA extraction, amplification, and sequencing—DNA was isolated from dried herbarium material following standard protocols using miniprep procedures employing 2× CTAB lysis buffer (2% CTAB = cetyltrimethylammoniumbromide, 100 mmol/L Tris pH 8, 20 mmol/L Na₂EDTA, 1.4 mol/L NaCl) (Zolan and Pukkila, 1986). Samples of dried basidiome fragments were homogenized with a plastic pestle in a microcentrifuge tube (1.5 mL) with 300–500 μL 2× CTAB lysis buffer. After soaking for several hours, tubes were incubated in 70°C for 30 min. An equal volume of chloroform was added,

and the tubes were vortexed and centrifuged at 10000 g for 15 min. The aqueous phase was transferred to a new tube and purified either by ethanol/isopropanol precipitation or GeneClean no. III (Bio 101, La Jolla, California, USA) and resuspended in 55 μL ddH₂O. The DNA extracts were diluted to 0.1–1.0 ng/μL for use in the polymerase chain reactions (PCR).

Polymerase chain reaction amplifications (PCR) followed the protocol of Vilgalys and Hester (1990) in a final volume of 25 μL. Primers ITS1 and ITS4 (White et al., 1990) were used for PCR amplification and sequencing of the internal transcribed spacers from the ribosomal genes. Reactions were performed in a Perkin Elmer 480 thermocycler (Perkin Elmer, Foster City, California, USA).

Amplified PCR products were quantified by gel electrophoresis on a 0.8% agarose gel stained with ethidium bromide and purified by microfiltration using Ultrafree-MC centrifugal columns (Millipore, Bedford, Massachusetts, USA). Sequencing was performed by fluorescent dye terminator chemistries following the manufacturer's instructions (Perkin Elmer) with automated sequencers (ABI 3700 or ABI 377, Perkin-Elmer, Norwalk, Connecticut, USA). Sequence chromatograms were compiled with Sequencher software, version 2.0 (Gene Codes, Ann Arbor, Michigan, USA). Sequences from this study have been submitted to GenBank.

Sequence alignments—Sequences were aligned by hand in the text editor of PAUP* version 4.0D64 (Swofford, 1998). Four sequence alignments were generated. The first alignment was primarily used to define the major clades, which were then analyzed separately. It included 151 sequences representing the 114 taxa under investigation. After the introduction of gaps, the alignment included 859 nucleotide positions. Several areas of ambiguous alignment were excluded. From the analysis of the 507 unambiguously aligned characters, 207 characters were constant, 49 were parsimony uninformative, and 251 characters were parsimony informative. To correspond to the dominant species in the three major clades, these three clades will be referred to as the *Cortinarius* clade, the *Hebeloma/Hymenogaster/Naucoria* clade, and *Descolea* clade. To distinguish between clade names and names of taxa, the former are not italicized.

The second alignment was made for 81 ingroup taxa belonging to the largest clade, the *Cortinarius* clade, using three *Hebeloma* spp. as outgroup. The alignment of those 106 sequences was 859 nucleotide positions long; 555 characters remained after excluding gapped and ambiguous areas; 266 characters were constant, 58 were parsimony uninformative, and 231 included characters that were parsimony informative.

The third alignment included the *Hebeloma/Hymenogaster/Naucoria* clade, consisting of 29 sequences for 11 species of *Hymenogaster*, 7 species of *Hebeloma*, and 3 species of *Naucoria*. Four *Cortinarius* spp. and three *Gymnopilus* spp. were used as outgroup. The alignment was 859 nucleotide positions long; 556 characters were included, of which 318 characters were

constant, 68 parsimony uninformative, and 170 characters parsimony informative.

The fourth alignment included 31 sequences belonging to the *Descolea* clade including eight taxa of *Descolea*, two taxa of *Descomyces*, two taxa of *Setchelliogaster*, four *Cortinarius* spp., and three *Hebeloma* spp. This alignment was 859 nucleotide positions long, of which 534 remained after excluding gaps/areas of ambiguity and of which 345 characters were constant, 73 were parsimony uninformative, and 116 characters were parsimony informative. Alignments are available from TreeBASE (accession number S636: M988–990).

Phylogenetic analyses—Phylogenetic analyses were performed in PAUP* version 4.0D64 (Swofford, 1998). The first alignment was analyzed by maximum parsimony (MP) with the following settings: MULPARS on, steepest descent not in effect, MAXTREES set to 100, equal character weights, gaps treated as missing. The most parsimonious trees were searched with tree-bisection-reconnection (TBR) branch swapping. Starting trees were obtained by random-sequence addition; 100 heuristic searches were performed, and the shortest trees over all replicates were kept and assumed to be the most parsimonious reconstructions. Support for branches was evaluated using the bootstrap method (heuristic search; bootstrap replicates = 100; addition sequence = random; TBR on, MAXTREES = 100 in each replicate). Three major clades were well supported and were used to generate the alignments two, three, and four. Each of those latter alignments was analyzed separately, which allowed the inclusion of more unambiguous positions.

Maximum parsimony (MP) was employed for analysis of alignment two with the PAUP* settings as above, but MAXTREES was set to 25000. Topological constraints were employed to investigate alternative tree topologies (monophyly of *Protoglossum* and *Thaxterogaster*). To test whether constrained topologies were significantly worse, these were compared to the unconstrained trees using the nonparametric comparison test of Templeton (Templeton, 1983) and the Kishino-Hasegawa test (Kishino and Hasegawa, 1989) as implemented by PAUP*. Maximum-likelihood ratio tests (LRT) (Goldman, 1993; Huelsenbeck and Rannala, 1997) were employed in order to identify a simple and robust substitution model for each data set. The LRT were conducted from trees obtained in the MP analyses. After model estimation, one MP tree was used as a starting tree for TBR searches by use of maximum likelihood (ML) with parameters derived from the model fitting. The ML search was allowed to proceed for 85 h. Bootstrap support for branches obtained in ML searches were estimated with 2000 replicates of the “fast bootstrap” option in PAUP* using parsimony (MULPARS off and no branch swapping).

For alignment three, the *Hebeloma/Hymenogaster/Naucoria* clade, MP was employed as described above. Two hundred heuristic searches were performed, and the shortest trees were kept and assumed to be the most parsimonious reconstructions. These 35 trees were used as starting trees for an additional heuristic search with TBR and a reconnection limit of 27, MAXTREES = 5000. Support for each branch was estimated by 200 bootstrap replicates (MULPARS off and no branch swapping) (Felsenstein, 1985). Topological constraints were employed to test the monophyly of *Hymenogaster*, *Hebeloma*, and *Naucoria*. After model estimation for ML analysis, one MP tree was used as starting tree for TBR searches using parameters derived from the LRT. Bootstrap supports for branches obtained in ML searches were estimated with 100 random bootstrap replicates under parsimony.

RESULTS

Analysis of the complete data set—The 100 best trees kept from 100 random heuristic searches of the 151 sequences included in the first alignment were used to produce the tree shown in Fig. 4 (tree score = 1426, consistency index [CI] = 0.3805, retention index [RI] = 0.8175). Taxa fell into three major clades that were all highly supported by bootstrap values, the *Cortinarius* clade, the *Hebeloma/Hymenogaster/Naucoria* clade, and the *Descolea* clade.

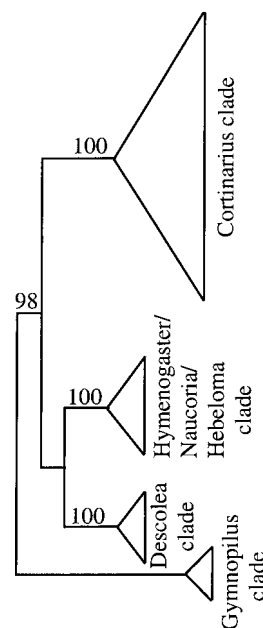


Fig. 4. The investigated 114 taxa fall into three major clades identified as the *Cortinarius* clade, the *Hebeloma/Hymenogaster/Naucoria* clade, and the *Descolea* clade. *Gymnopilus* was used as outgroup to root the phylogeny. This overview is based on the strict consensus of 100 equally parsimonious trees. Support for clades is indicated by bootstrap values above branches.

Analysis of individual data sets: the *Cortinarius* clade—Parsimony analysis of the 106 sequences included in the *Cortinarius* clade yielded >20000 equally parsimonious trees (tree score = 1034 steps, CI = 0.4516, homoplasy index [HI] = 0.5484, RI = 0.7080, rescaled consistency index [RC] = 0.3198). The best fitting ML model employed the Hasegawa-Kishino-Yano model (HKY) (Hasegawa, Kishino, and Yano, 1985) with rate heterogeneity, transition/transversion ratio = 1.9548. The model accounted for among-site rate variation with a gamma-shape parameter estimation of 1.302271, and four rate categories represented by mean. Assumed nucleotide frequencies were A = 0.24660, C = 0.19754, G = 0.22165, T = 0.33421. Number of substitution types = 2. The overall topology of the strict consensus tree of the MP analysis (not shown) corresponds with the ML tree ($-\ln(\text{likelihood}) = 6306.30371$) (Fig. 5).

All outgroup taxa formed a monophyletic group including the agaricoid *Cortinarius*, *Dermocybe*, *Rozites*, and *Cuphocybe*, and the sequestrate *Thaxterogaster*, *Protoglossum*, *Quadrispora*, and two species of *Hymenogaster*. The basal phylogenetic relationships are not strongly supported. However, all searches found 19 clades with bootstrap support >50%, which correspond to parts of classical subgenera or sections of *Cortinarius*, and to *Dermocybe*, *Rozites*, and *Cuphocybe* (which are nested within the *Cortinarius* clade). For purposes of discussion, we address the relationships of sequestrate forms by naming the subclades of *Cortinarius* with the subgeneric and sectional nomenclature in current use.

Bootstrap support for larger monophyletic groups (>3 taxa) within the *Cortinarius* clade was obtained for *Dermocybe* and for parts of the subgenera *Telamonia*, *Phlegmacium*, *Myxaciium*. Three lineages of *Telamonia* spp. were found: *Telamonia* I, *Telamonia* II, and *Myxotelamonia*. *Phlegmacium* spp. group into four subclades, and *Myxaciium* spp. into three. Several

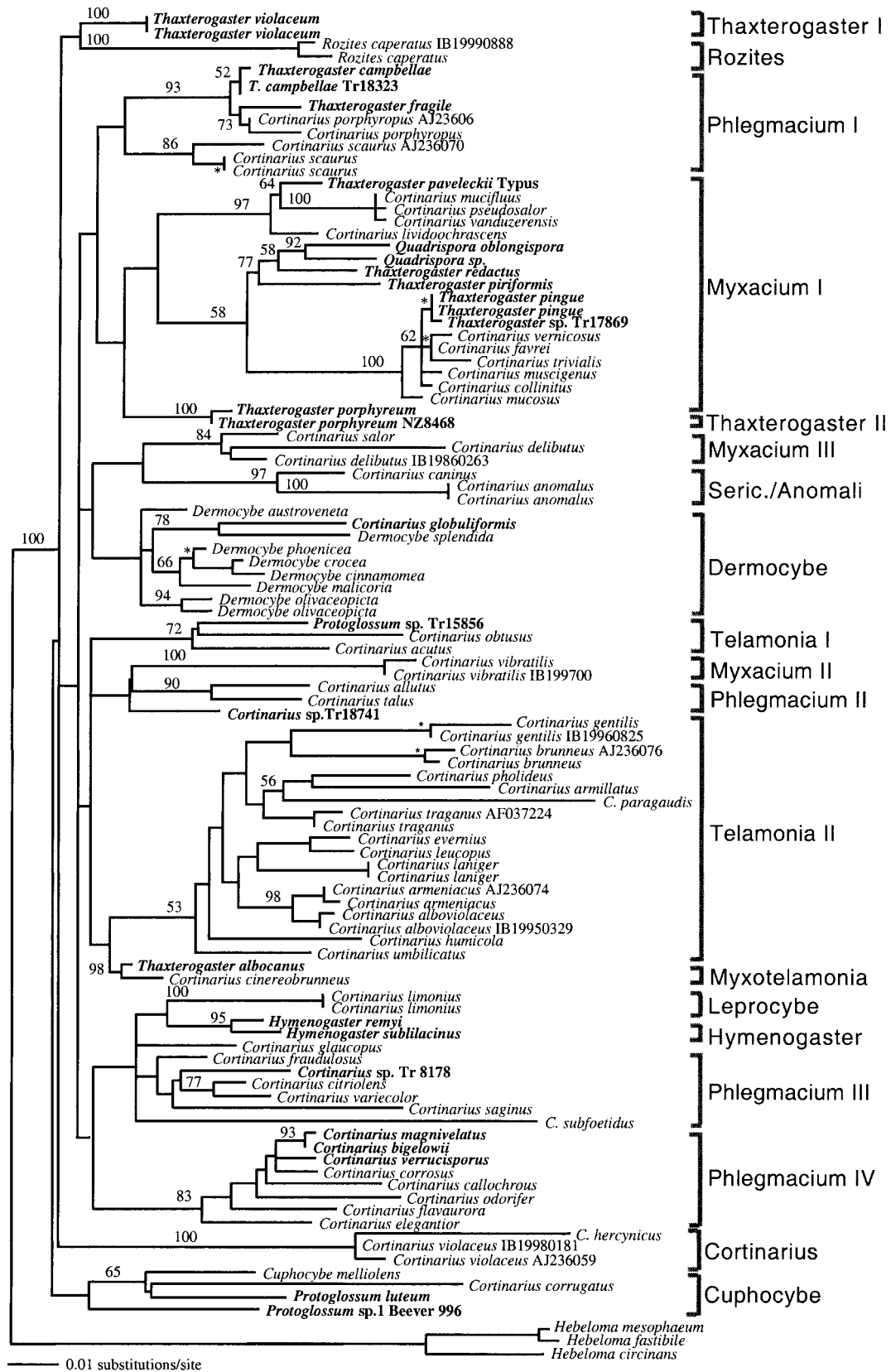


Fig. 5. Phylogenetic relationships (maximum likelihood) in the Cortinariaceae. Values above branches indicate bootstrap supports calculated via maximum parsimony. Asterisks indicate bootstrap values >50%. Sequestrate taxa are written in boldface type.

TABLE 1. Tree statistics for the *Cortinarius* clade: comparison of unconstrained trees and trees constrained for monophyly of *Thaxterogaster* and *Protoglossum*. Asterisked values in table indicate significance at $P < 0.05$.

	No constraints	<i>Thaxterogaster</i>	<i>Protoglossum</i>
Tree length	1034	1095	1065
Consistency index	0.452	0.426	0.438
Retention index	0.708	0.677	0.692
Kishino-Hasegawa test	best trees	<0.0001*	<0.001*
Templeton test	best trees	<0.0001*	<0.001*

Sericeocybe taxa of section *Anomali* also form a clade, with remaining species grouped into *Telamonia* II. Species currently classified in the subgenus *Leprocybe* are found in different clades.

Placement of sequestrate taxa related to *Cortinarius*

Twenty-two sequestrate taxa were placed with bootstrap support >50% into nine clades together with agaric-like forms (Fig. 5). However, two *Hymenogaster* spp., *Thaxterogaster violaceum*, *T. porphyreum*, *Protoglossum* sp. Beever 996, and two sequestrate *Cortinarius* spp. (Tr18741, Tr8178) could not be clearly related to any of the >400 sequences in the current ITS database of *Cortinarius* and related genera (U. Peintner, unpublished data).

Searches with topological constraints for the monophyly of *Thaxterogaster* all yielded longer (i.e., less parsimonious) trees than searches without constraints. Constrained trees were all clearly rejected by the Templeton and the Kishino-Hasegawa tests (Table 1) indicating that *Thaxterogaster* is not monophyletic. The species currently classified in the genus *Thaxterogaster* have multiple origins: (1) the relationships of clades *Thaxterogaster* I and II are not known; and (2) the other *Thaxterogaster* spp. are related to agaricoid taxa of the three clades Phlegmacium I, Myxacium I, and Myxotelamonia (Fig. 5).

Additionally, the monophyly of *Protoglossum* spp. was rejected (Table 1); the type of the genus *P. luteum* and possibly also *Protoglossum* sp. Beever 996 are related to taxa of the clade *Cuphocybe*, while *Protoglossum* sp. Tr15856 is related to *Telamonia* I.

Sequestrate *Cortinarius* spp. are found within the Phlegmacium III, IV, and *Dermocybe* clades. *Cortinarius* Tr8178 is nested within Phlegmacium III and is a sistergroup to *C. varicolor* and *C. citriolens*. Three partly hypogeous *cortinari*, *C. magnivelatus*, *C. bigelowii*, and *C. verrucisporus*, are nested within Phlegmacium IV. *C. globuliformis* is closely related to *Dermocybe splendida*.

Two taxa of *Quadrispora* tested form a clade closely related to *T. redactus* and *T. piriformis* and thus are a purely sequestrate subclade of Myxacium I. Furthermore, *Hymenogaster renyi* and *H. sublilacinus* form their own small *Hymenogaster* clade within the *Cortinarius* clade.

Analysis of individual data sets: the *Hebeloma/Hymenogaster/Naucoria* clade—The heuristic searches yielded 18 best trees with a score of 491, CI = 0.6680, HI = 0.3320, RI = 0.7504. The best fitting ML model for this alignment was as follows: assumed nucleotide frequencies: A = 0.25515, C = 0.20651, G = 0.20895, T = 0.32939; number of substitution types = 6; proportion of invariable sites: 0.49987; rates follow a gamma distribution with shape parameter 2.94413, four rate categories. This corresponds to the general time-reversible model (Yang, 1994) with rate heteroge-

neity. The tree resulting from the ML analysis ($-\ln(\text{likelihood}) = 3262.13834$) (Fig. 6) was completely congruent with the MP trees (not shown). The *Hebeloma/Hymenogaster/Naucoria* clade is a monophyletic group consisting of five well-supported clades: (1) the *Hebeloma* clade; (2) two clades of *Naucoria* representing the two currently accepted subgenera of the genus, subgenus *Submelinoideae* and subgenus *Naucoria*; and (3) two clades of *Hymenogaster*, with the clade *Hymenogaster* I including the type species *H. bulliardii* and clade *Hymenogaster* II. The deeper branches of each clade were not resolved. All trees enforcing the monophyly of *Hebeloma* and/or *Hymenogaster* had the same length than all unconstrained trees, indicating that monophyly for *Hymenogaster* cannot be rejected from our data. All trees with constraints for monophyly of *Naucoria* were five steps longer than unconstrained trees; nevertheless, a monophyletic *Naucoria* could not be rejected by the Kishino-Hasegawa test or by the Templeton test (Table 2).

Analysis of individual data sets: the *Descolea* clade—Four MP trees of 257 steps were obtained (CI = 0.650, RI = 0.847). The best fitting ML model for this alignment was as follows: assumed nucleotide frequencies: A = 0.24621, C = 0.21166, G = 0.20862, T = 0.33351; number of substitution types = 6; proportion of invariable sites: 0.30724; rates follow a gamma distribution with shape parameter 1.26766, four rate categories. This corresponds to the general time-reversible model (Yang, 1994) with rate heterogeneity. The tree resulting from the ML analysis ($-\ln(\text{likelihood}) = 3191.96939$) (Fig. 7) was congruent with the MP trees (not shown). The Kishino-Hasegawa and the Templeton tests clearly reject monophyly of *Setchelliogaster*, while the monophyly of *Descomyces* is congruent with all unconstrained trees.

The *Descolea* clade includes the secotioid genus *Setchelliogaster* and the gastroid genus *Descomyces*. The two *Descomyces* spp. form a monophyletic group and share a common ancestor with *Descolea antarctica*. A second subclade includes *Descolea recedens*, *D. gunnii*, *D. maculata*, and *S. australiensis*. Also *S. tenuipes* and *D. rheophylla* collections form their own subclade, which is characterized by a striking combination of long and short branches. In contrast, within collections of *S. australiensis* the branch lengths are comparatively homogenous (Fig. 7). *Descolea rheophylla* has recently been synonymized with *S. tenuipes* (Lago, Bougher, and Castro, 2000). However, for practical reasons, in this paper we will continue to use this name for the agaricoid form of *S. tenuipes*.

DISCUSSION

The current classification of *Cortinarius*—The great variety of forms and groups of the large agaricoid genus *Cortinarius* has caused problems in delimiting subgenera within this

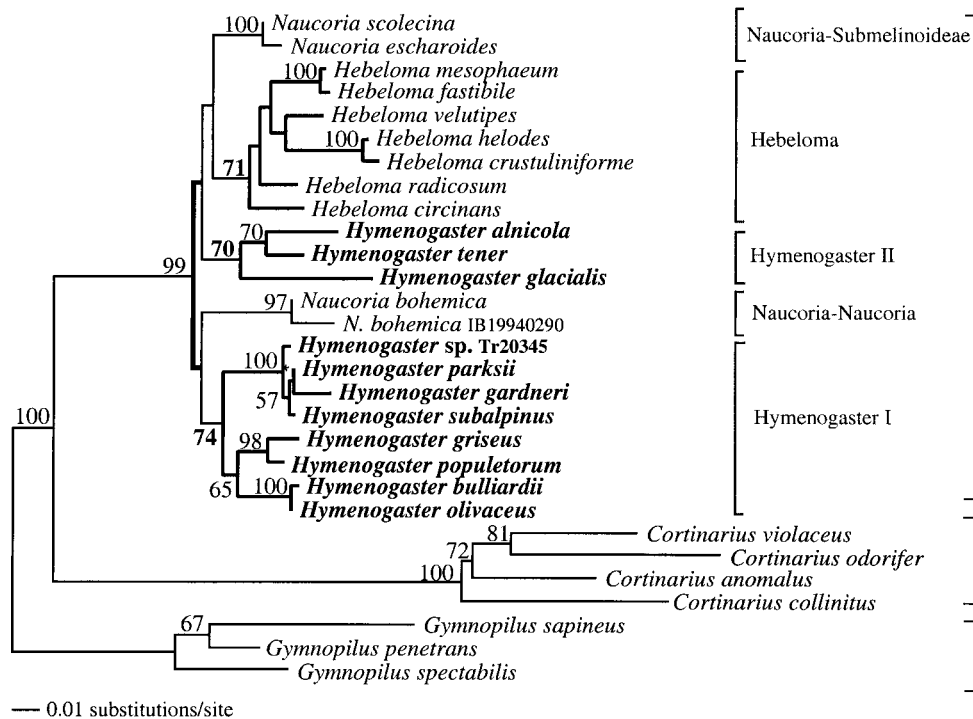


Fig. 6. Phylogram of the Hebeloma/Hymenogaster/Naucoria clade resulting from maximum likelihood analysis ($-\ln(\text{likelihood}) = 3262.13834$). Sequestrate taxa are written in boldface type. Five well-supported clades can be recognized: *Hebeloma*; two clades of *Naucoria*, representing the two subgenera *Submelinoideae* and *Naucoria*; and two clades of *Hymenogaster*: Hymenogaster I, including the type species *H. bulliardii*, and Hymenogaster II. *Cortinarius* and *Gymnopilus* were used as outgroups to root the phylogeny. Bootstrap values $>60\%$ are shown above or left of branches; an asterisk indicates a bootstrap value of 65%.

species-rich complex, as well as separating the genus *Cortinarius* from closely related genera such as *Rozites* and *Cuphocybe*. Under current taxonomy (Moser and Horak, 1975; Moser, 1983; Hansen and Knudsen, 1992), eight subgenera of *Cortinarius* are recognized: *Cortinarius*, *Cystogenes*, *Dermocybe*, *Leprococybe*, *Myxaciium*, *Paramyxaciium*, *Phlegmacium*, *Sericeocybe*, and *Telamonia*. This study and others (Liu, Rogers, and Ammirati, 1997; Hoiland and Holst Jensen, 2000; Seidl, 2000; Seidl et al., 2000) indicate that these subgenera do not correspond to monophyletic groups. Therefore, a reclassification of *Cortinarius* into natural units would be appropriate, but is beyond the scope of this paper.

Unsettled family affinities: the genera around *Cortinarius*—The question of “family affinities” of *Cortinarius* and other brown-spored genera of Agaricales is still unsettled (Hibbett and Thorne, 2000; Moncalvo et al., 2000). Based on alignability of ITS sequences, a close relationship between *Cortinarius*, *Hebeloma*, *Naucoria*, and *Descolea* can be assumed. Based on the same criterion, the genus *Gymno-*

pilus Karst. was chosen as the outgroup. This genus is suitable as an outgroup as the sampled species form a monophyletic clade, are saprobic in contrast to the ectomycorrhizal ingroup taxa, and no sequestrate relatives to *Gymnopilus* have been reported at present.

Internal transcribed spacer sequences of *Inocybe*, *Callistosporium*, *Laccaria*, and *Leucopaxillus* were also examined to serve as outgroup to root the *Cortinarius* phylogeny, as suggested by classical taxonomy (Singer, 1975, 1986), chemotaxonomy, or previous molecular studies (Moncalvo et al., 2000). (These sequences have also been deposited in GenBank.) However, none of these sequences were alignable with our ingroup data set; therefore, a close relationship of the above genera with *Cortinarius*, *Hebeloma*, *Naucoria*, *Descolea*, and *Gymnopilus* can be excluded. These preliminary results are particularly interesting when higher taxonomic categories, e.g., the delimitation of the family Cortinariaceae, are addressed, but more extensive molecular studies are needed to resolve these questions.

The puzzling phylogeny of the *Cortinarius* clade—The

TABLE 2. Comparison of tree statistics for constrained and unconstrained trees of the Hebeloma/Hymenogaster/Naucoria clade. Abbreviations for constraints to monophyly: HY = *Hymenogaster*, HE = *Hebeloma*, NA = *Naucoria*. Asterisked values in table indicate significance at $P < 0.05$.

	No constraints	HY	HE	NA	HE + NA	HY + HE + NA
Tree length	491	491	491	496	496	496
Consistency index	0.668	0.668	0.668	0.661	0.661	0.661
Retention index	0.750	0.750	0.750	0.743	0.743	0.743
Kishino-Hasegawa test	best	1.000	1.000	0.166	0.0956	0.0587
Templeton test	best	1.000	1.000	0.166	0.0956	0.0588

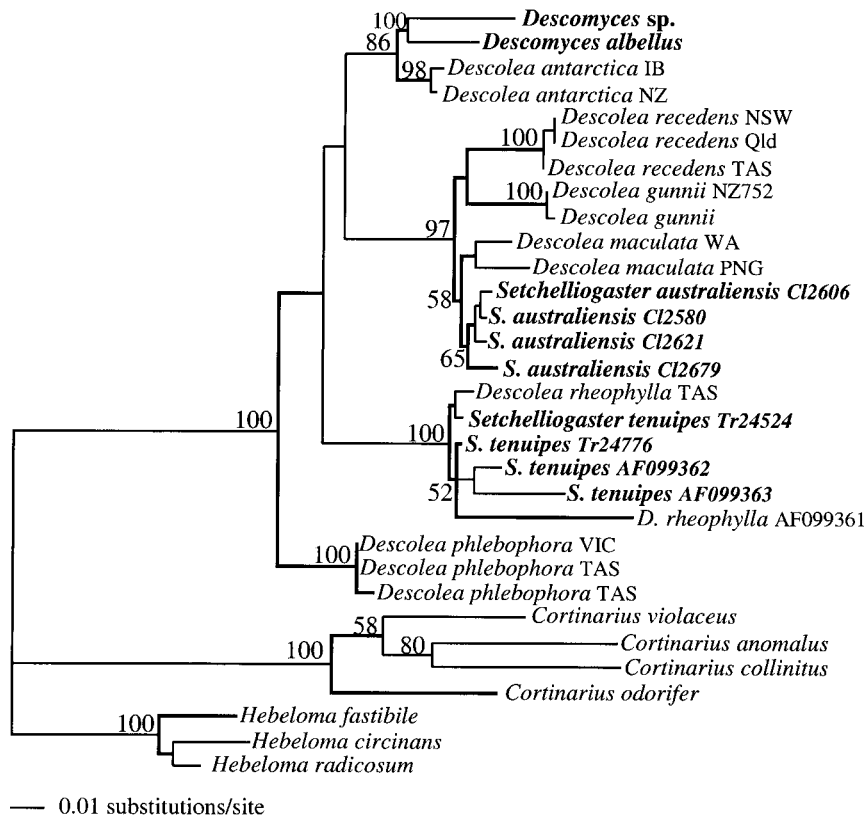


Fig. 7. Phylogram of the *Descolea* clade, resulting from maximum likelihood analysis [$-\ln(\text{likelihood}) = 3191.96939$]. Support for clades is indicated by bootstrap values above branches. Sequestrate taxa are written in boldface type.

Cortinarius clade is a large monophyletic group, which includes agaricoid taxa of the genera *Cortinarius*, *Dermocybe*, and *Rozites*. No premature conclusions should be drawn about the phylogeny of *Cuphocybe* based on one single taxon, as neither the type species (*Cuphocybe olivacea*) nor other species of *Cuphocybe* have been included in this study.

The deeper relationships within *Cortinarius* are not well resolved by ITS sequences (Fig. 5). However, our results indicate that except for the subgenera *Dermocybe* and *Cortinarius*, the currently recognized subgenera are polyphyletic. This result agrees with previous studies (Liu, Rogers, and Ammirati, 1997; Hoiland and Holst Jensen, 2000; Seidl, 2000).

The monophyletic *Dermocybe* clade nests within the *Cortinarius* clade, which consists of a well-supported core clade including most of the known boreal species of *Dermocybe* (Liu, Rogers, and Ammirati, 1997) and 2–3 smaller clades. The monophyletic subgenus *Cortinarius* consists of only few species, which are characterized by large violaceous basidiomes with cheilo- and pleurocystidia.

Many taxa currently classified in the subgenus *Telamonia* also form a monophyletic group. Although we cannot confirm a consistent subdivision of *Cortinarius* into the two lineages *Telamonia* II and *Cortinarius* sensu stricto (s.s.) as proposed by Hoiland and Holst Jensen (2000), the apparent lack of sequestrate forms may support the isolated position of *Telamonia* II within the *Cortinarius* clade. However, a few species currently assigned to this subgenus are outside the core clade and show relationships to sequestrate taxa: *C. acutus* and *C. obtusus* (*Telamonia* I), as well as the type species of section *Myxotelamonia*, *C. cinereobrunneus*.

Subgenus *Myxacium* is polyphyletic, comprising at least three distinct lineages: *Myxacium* I, including the monophyletic sections *Defibulati* and *Myxacium*; *Myxacium* II (*Cortinarius vibratilis* = section *Ochroleuci*); and *Myxacium* III (*C. salor* and *C. delibutus* = section *Delibuti*). Interestingly, sequestrate taxa seem to be related only to *Myxacium* I.

The morphology-based classification of sequestrate fungi related to *Cortinarius*—Many sequestrate genera have been placed in the *Cortinariaceae* based on shared characters such as brown, verrucose spores, a nongelatinized trama, an array of specific pigments, and the ectomycorrhizal habit. The sequestrate forms with the highest morphological similarity with agarics are the emergent species of *Cortinarius* (Thiers and Smith, 1969; Watling, 1980; Bougher and Malajczuk, 1986; Fogel, 1994). The gills of these fungi are radially arranged and produce a spore deposit. Unlike typical agaricoid species of *Cortinarius*, however, the stipe is short and the gills often disintegrate with age while the partial veil commonly remains unbroken at maturity.

The next step of morphological reduction occurs within the genus *Thaxterogaster* (Singer, 1951); taxa classified in this genus are characterized by basidiomes with a reduced to well-developed stipe and a basally exposed hymenium with a loculate to lamellate gleba (Fig. 2).

Hymenogaster Vittadini was widely considered as “end-point phylogenetic position in the *Cortinariaceae* presenting a truly hypogeous member” (Fig. 3) (Smith, 1966; Beaton, Pegler, and Young, 1985; Fogel, 1985). However, as Bougher and Castellano (1993) discussed at length, the generic concept of

Hymenogaster sensu lato (s.l.) has been inflated beyond the original circumscription intended by Vittadini. Therefore, the authors recombined taxa from *Hymenogaster* s.l. into several genera, e.g., *Destuntzia* (Fogel, 1985), *Descomyces*, *Quadrispora*, *Gautieria*, *Timgrovea*, and *Protoglossum* (formerly *Cortinomyces*; May, 1995).

As redefined by Bougher and Castellano (1993), *Hymenogaster* s.s. is restricted to taxa with thick-walled, ellipsoid to fusiform spores bearing a large, cup-like hilar appendix, as in the type species *H. bulliardii*. Because such spores are very different from those of the genus *Cortinarius*, the relationship of *Hymenogaster* s.s. to other established taxa of the Agaricales remained unclear (Bougher and Castellano, 1993).

Hymenogaster spp. with warty spores and closely adhering epispodium, which closely resemble spores of *Cortinarius* spp., were recombined into the genus *Protoglossum* (type: *P. luteum*) (Bougher and Castellano, 1993). As distinguished from *Thaxterogaster* spp., *Protoglossum* spp. have gastroid basidiomes without stipes or with very reduced stipes (columella). However, in species with highly variable development of columella (e.g., *T. luteirufescens*), boundaries between *Thaxterogaster* and *Protoglossum* are indistinct (Bougher, 1997; Bougher and Syme, 1998).

Quadrispora spp. are characterized by hypogeous basidiomes with a viscid peridium and irregularly warted statismospores, which adhere to each other in persistent tetrads after release from the basidium. The type of the genus is *Q. oblongispora*. The relationship of *Quadrispora* to other taxa of the Agaricales was not previously known (Bougher and Castellano, 1993).

Descomyces was segregated from *Hymenogaster* because of the distinct spore morphology and peridium structure resembling *Setchelliogaster* and *Descolea*. In contrast to the secotoid *Setchelliogaster*, the basidiomes of *Descomyces* are gastroid. The type of the genus is *Descomyces albus* (Bougher and Castellano, 1993).

Setchelliogaster accommodates taxa with a very reduced to absent stipe and basally exposed hymenophore. The key characters for this genus are a peridial surface consisting of inflated, isodiametric elements, and pale brown basidiospores with finely verruculose ornamentation overlaid by a hyaline myxosporium (type: *S. tenuipes*). Significant evidence for the close relationship between *Setchelliogaster* and *Descolea* was based on the observation that both genera can produce a variety of basidome morphologies, which transgress the formal taxonomic definitions of the genus (Martin and Roccabruna, 1999; Lago, Bougher, and Castro, 2000).

Multiple origins of sequestrate fungi from ectomycorrhizal ancestors—Sequestrate forms occur in all three major ectomycorrhizal lineages of Cortinariaceae (the *Cortinarius*, *Hebeloma*/*Hymenogaster*/*Naucoria*, and *Descolea* clades) but not within the saprobic *Gymnopilus* (Figs. 4–7). These results indicate multiple origins of sequestrate forms from ectomycorrhizal ancestors. Further evidence for this hypothesis arises from the fact that animals play an important role in dispersing spores of the generally obligately ectomycorrhizal, sequestrate fungi (Claridge, Castellano, and Trappe, 1996). The production of spores at the root zone and the maintenance of spores in the soil for long periods are generally regarded as evolutionary advantages for mycorrhizal fungi (Fogel, 1992; Miller, Torres, and McClean, 1994). Long-distance dispersal is effected by

mycovores, and spores in the faeces are likely to be deposited near suitable plant roots (Bougher and Lebel, 2001).

Molecular data support the segregation of *Hymenogaster* s.l. in distinct, independently derived lineages as proposed by Bougher and Castellano (1993). Furthermore, ITS phylogeny also places these lineages in relationship to their agaricoid counterparts. Hence, only sequestrate *Cortinarius* spp., *Thaxterogaster*, *Quadrispora*, *Protoglossum*, and two *Hymenogaster* spp. (*H. remyi*, *H. sublilacinus*) share common ancestors with *Cortinarius* taxa. The other *Hymenogaster* taxa under investigation share a common ancestor with *Hebeloma* and *Naucoria*. *Setchelliogaster* and *Descomyces* are derived from *Descolea*, the former being polyphyletic and the latter monophyletic.

Sequestrate fungi within the *Cortinarius* clade: polyphyletic origin vs. radiation into monophyletic entities—

Three different sequestrate basidiome morphologies (emergent, secotoid, and gastroid) occur within the *Cortinarius* clade (Figs. 1–3 and 5): emergent *Cortinarius* spp. are strikingly morphologically similar to their agaric-like relatives. The sequestrate *Cortinarius* spp. related to Phlegmacium IV have a strongly developed white veil like their agaric relatives, but in the most derived taxon *C. magnivelatus*, the pileus is dry. In addition *C. globuliformis* is related to *Dermocybe splendida* and has bright yellow colors on the cap, gills, stipe, and the surrounding matlike masses of mycelium (Bougher and Malajczuk, 1986), a typical character for many species of *Dermocybe*. A unique combination of anthraquinone derivatives has been found in *D. splendida* (Horak, 1987; Gill, 1995). Anthraquinone pigments have been proven to be a very useful character in *Dermocybe* taxonomy, but they have not yet been investigated in sequestrate *Cortinarius* spp., though this may be a simple tool to test relationships in the future. As further indicated by other relationships found in this study, it might also be useful to apply other macrochemical tests currently used for *Cortinarii* (e.g., potassium hydroxide [KOH] 30% reaction) on sequestrate forms as well. Similarly, testing the fluorescence behavior of basidiomes under UV light might be worthwhile (Moser, 1960, 1983).

Our results clearly demonstrate that emergent *Cortinarius* taxa evolved from agaric-like precursors, but also indicate that once successful sequestrate forms are established, they tend to radiate independently from their agaric relatives (e.g., the clade *C. magnivelatus*, *C. bigelowii*, and *C. verrucisporus*). This confirms the hypothesis proposed by Fogel (1994) of a recent parallel evolution of emergent *Cortinarius* taxa based on distribution data.

The sequestrate *Thaxterogaster* spp. have multiple origins within the *Cortinarius* clade: *T. albocanus* is closely related to *Cortinarius cinereobrunneus*, a species belonging to section *Myxotelamonium* resembling typical *Telamonium* spp., but having a gelatinized epicutis or veil. *Thaxterogaster campbellae* and *T. fragile* are derived from taxa of clade Phlegmacium I, whereas *T. violaceum* has unknown relationships. However, our results indicate that most *Thaxterogaster* taxa could be related to clade Myxacium I: *T. pingue* and *Thaxterogaster* sp. Tr17864 form a well-supported clade as sister group of Myxacium I, sect. *Myxacium* (*C. trivialis*, etc.) (Fig. 5). Key characters of this section are presence of clamps and spores >10 μ m. *Thaxterogaster redactus* and *T. piriformis* share a common ancestor with all these above-mentioned taxa.

Thaxterogaster paveleckii, with thick, slimy peridium and

no clamp connections, is closely related to Myxaciium I, section *Defibulati* (*C. mucifluus*, etc.). This secotioid species exhibits the main synapomorphy supporting this section *Defibulati*, namely the complete lack of clamp connections. The lack of clamp connections in *T. porphyreum* can be interpreted as a further indication for its affinities to Myxaciium I, as clamp connections usually occur in *Cortinarius*. Hence, Myxaciium I seems to be a "hot spot" of evolution of *Thaxterogaster*-like forms, including also the development of the gastroid *Quadrispora* taxa (Fig. 5).

Two other gastroid genera are related to *Cortinarius*, namely *Protoglossum* and two *Hymenogaster* spp., which are primarily delimited from each other based on spore morphology. *Hymenogaster sublilacinus* and *H. remyi* have no natural relationships to *Hymenogaster* s.s. (Figs. 5–6). They belong to a distinct group of species with small, nonbeaked spores. Such species have been placed in the subgenus *Dendrogaster* (formerly genus *Dendrogaster* Bucholtz). Unfortunately, the type species of *Dendrogaster*, *D. connectens* Bucholtz, has beaked spores and thus does not belong to this species complex, but is a synonym of *Hymenogaster* (Fogel, 1985). The necessary nomenclatural changes to accommodate these species will be published in a forthcoming paper.

Overall, each of the different sequestrate basidiome forms related to *Cortinarius* appears in various subclades, clearly indicating multiple origins. However, certain sequestrate forms seem to be more frequent in certain groups, e.g., *Thaxterogaster* in Myxaciium I and emergent *Cortinarius* spp. in clades of Phlegmacium. This observation might indicate an increased susceptibility of certain groups to develop a particular sequestrate form. Furthermore, this might also be interpreted as an indication for relationships between clades sharing similar sequestrate forms, e.g., between clades Phlegmacium II, III, and IV.

Although several types of sequestrate forms occur within the *Cortinarius* clade, these forms are phylogenetically not closely related with each other: only *Thaxterogaster* and *Quadrispora* can be found in the same clade, indicating that gastroid taxa could possibly derive from secotioid forms. However, *Quadrispora* could also share a common ancestor with *Thaxterogaster*. This implies that the different sequestrate basidiome forms can evolve independently from each other without intermediate forms.

When several sequestrate taxa occur in a clade, they tend to form a sister group to the agaric-like forms rather than being nested within them. Thus, although sharing a common ancestor, they evolve and speciate separately. This pattern of evolution is especially striking in Myxaciium I, in which a sampling bias for agaric-like forms can be excluded. We conclude that sequestrate forms are of polyphyletic origin within the *Cortinarius* clade, but some of them are developing into monophyletic, purely sequestrate groups.

Overall, ITS phylogeny indicates a derived, recent radiation of sequestrate forms in *Cortinarius*. The low level of sequence divergence within the *Cortinarius* clade and *Hebeloma* (Aanen et al., 2000) suggests that these fungi have a relatively recent origin, with one to several periods of rapid speciation.

Is *Hymenogaster* polyphyletic?—*Hebeloma*, *Naucoria*, and *Hymenogaster* form a well-supported monophyletic clade (Fig. 6). Basal nodes within the *Hebeloma*/*Hymenogaster*/*Naucoria* clade are not well resolved. In any case, *Hymenogaster* has a common ancestor with both *Naucoria* and *Hebeloma*.

Hebeloma is monophyletic. Small *Hebeloma* spp. could be confused with *Naucoria* spp. but the latter never have a gelatinized epicutis and are exclusively ectomycorrhizal with *Alnus* (or more rarely, *Salix*). Because of the somewhat difficult delimitation, Kühner (1980) has proposed fusing *Hebeloma* and *Naucoria*. Our results confirm earlier data (Aanen et al., 2000), indicating that *Naucoria* is phylogenetically distinct from *Hebeloma*, which implies that the strict host preference of *Naucoria* has acted as a strong selection pressure. The two clades of *Naucoria* correspond to the two currently recognized sections: section *Naucoria* (*N. escharoides*, *N. scolecina*) with narrow cheilocystidia tapering to a subacute or acute apex, and section *Submelinoideae* (*N. bohémica*) with broad, nonattenuate cheilocystidia. The monophyly of *Naucoria* could not be rejected.

Also the monophyly of *Hymenogaster* could not be rejected. *Hymenogaster* I includes the type species *H. bulliardii* and others (Fig. 6), while *Hymenogaster* II consists of *H. alnicola*, *H. glacialis*, and *H. tener*. However, more extensive studies are needed to assess whether *Hymenogaster* is monophyletic, having achieved its special morphology only once.

The morphological plasticity of sequestrate taxa related to *Descolea*—Internal transcribed spacer data clearly show that *Descolea*, *Setchelliogaster*, and *Descomyces* form a distinct clade (Fig. 7) in which three types of basidiomes are present: *Descolea*, with agaric-like basidiomes, *Setchelliogaster*, with secotioid, sublamellate basidiomes, and *Descomyces*, with gastroid basidiomes and a loculate hymenium.

A broad variation in basidiome morphology has been observed for many species of *Descolea* and *Setchelliogaster* (Martin and Roccabruna, 1999; Lago, Bougher, and Castro, 2000). For instance, all transitional stages between agaricoid and secotioid fruit bodies have been observed for *D. rheophylla*, which therefore was synonymized with *S. tenuipes* (Lago, Bougher, and Castro, 2000). Based on morphological and molecular data, Martin and Roccabruna (1999) argue that *S. tenuipes* is recently derived from *D. rheophylla*. However, our molecular data indicate that the agaricoid morphology was rather reestablished from a secotioid form, in which the basidiome development of present-day individuals remains very plastic.

Setchelliogaster australiensis exhibits less morphological plasticity (variation) than *S. tenuipes* (Lago, Bougher, and Castro, 2000). Basidiomes of this taxon are morphologically highly variable, but no agaricoid forms, as in *S. tenuipes*, have been observed. Furthermore, secotioid forms have also been reported to occur in *Descolea maculata* and *D. gunnii* (Lago, Bougher, and Castro, 2000). *Hydnangium sublamellatum* is another ectomycorrhizal fungus with *Eucalyptus*, in which different developmental patterns from agaricoid to gastroid can be expressed amongst and within single collections. Such highly morphologically plastic taxa are probably very recently derived from agaricoid precursors (Bougher, Tommerup, and Malajczuk, 1993).

High levels of morphological plasticity pose problems for circumscribing taxa on a morphological basis. Taxa defined by basidiomal form are useful in a broad practical sense, but may not be biologically distinct, especially when their boundaries are transgressed by a high morphological variability. In such cases, the application of a phylogenetic species concept as defined by Harrington and Rizzo (1999) seems most appropriate: they diagnose species as the smallest aggregation of popula-

tions with a common lineage that share unique, diagnosable phenotypic characters. Thus, collections belonging to the clade around *Setchelliogaster tenuipes* should be regarded as one morphologically very plastic species.

The lack of morphological plasticity of *Descomyces* is surprising, as *Descomyces* was regarded as the most reduced form in the phylogenetic series including *Setchelliogaster* and *Descolea*. According to our molecular data, one plausible scenario for the development of sequestrate forms related to *Descolea* is a two-step mutation: the first evolutionary event occurred at the basal node of the *Descolea* clade, which would explain the morphological plasticity of most of the investigated *Descolea* spp. Second, in *Descomyces*, an apparently stable morphology has evolved, forming a small, but distinct gastroid lineage. The stability of such gastroid morphologies implies that here the information for certain morphological pathways is not readily reexpressed or was definitely lost.

The extreme morphological changes between *Descolea* and *Descomyces* are analogous to the case of *Rhizopogon* and *Suillus*, in which striking macromorphological changes combined with low levels of genetic sequence divergence and extreme similarity in micromorphology have also been observed (Bruns et al., 1989). Hence, this is another case in which morphological change is not related to molecular change in the ITS region of rDNA.

Evolution of sequestrate forms—The gastroid lineages without intermediate secotioid forms, as well as the high diversity of apparently stable (i.e., persistent over time) secotioid forms, brings into question the severity of selection against intermediate forms hypothesized by previous molecular studies (Bruns et al., 1989; Baura, Szaro, and Bruns, 1992). Our results suggest instead several possible evolutionary scenarios for the development of sequestrate forms: (1) evolution of morphologically very plastic forms (*Setchelliogaster*) from gilled precursors; (2) evolution of stable emergent taxa (e.g., *Cortinarius magnivelatus* group) from gilled precursors; (3) evolution of stable secotioid taxa from gilled precursors (*Thaxterogaster*); (4) fast evolution of stable gastroid taxa from gilled precursors, with or without unstable intermediate forms (*Hymenogaster*, *Descomyces*); and (5) gradual evolution of gastroid forms from stable secotioid ancestors (*Quadrifera*?).

For morphologically very plastic taxa, it has been suggested that the different basidiome forms are due to differences in the expression of, or relative timing and duration of, genetic events controlling various key processes during development (Bougher and Lebel, 2001). *Descolea rheophylla* is a well-documented example for agaric-like forms, which can derive secondarily from morphologically very plastic sequestrate forms. Here the information for ballistospore development has not been lost, but has been suppressed.

This could indicate that the loss of important morphogenetic information probably occurs in a later stage in the evolution of sequestrate forms, although this is a key step in the development of stable, sequestrate species. Mutations conferring the loss of functional development may occur in only a few genes, therefore morphological reduction appears to be rapid relative to molecular changes, e.g., recessive alleles at a single locus can cause the formation of sequestrate forms (Hibbett, Tsuneda, and Murakami, 1994). Such mutations and others may easily occur independently from other mutations, thus explaining why sequestrate forms within *Cortinarius* have repeatedly evolved from agaric-like precursors. Transformations resulting

from a loss of genetic information usually cannot be reversed. Hence, we hypothesize that successfully adapted, stable sequestrate forms tend to radiate, but they can radiate only into species with the same form of sequestrate basidiomes or into further reduced basidiome types.

The utility of morphological characters to establish natural relationships—The hypothesis that gasteroid adaptations have arisen at successive levels of basidiomycete evolution was already proposed by Ingold (1965) based on spore morphology and spore dispersal. Molecular phylogenies support this hypothesis: at higher taxonomic levels within basidiomycetes, Hibbett et al. (1997) showed that puffballs and other forms of Gasteromycetes have been repeatedly derived across the Hymenomycetes. On a lower taxonomic level, our study clearly demonstrates that sequestrate taxa are also related to distinct clades of agaric-like *Cortinarius*, *Hebeloma*/*Hymenogaster*/*Naucoria*, and *Descolea*. This demonstrates that changes in morphology from agaricoid to gastroid with all transitional forms have been achieved independently much more frequently than previously suspected across all taxonomic levels.

Interestingly, as early as 1892 Rehsteiner already suggested a possible relationship of *Hymenogaster* with agarics based on their fruit body development (Rehsteiner, 1892, cited in Reijnders, 2000). Later, *Hymenogaster* was redefined and a close relationship of *Protoglossum* and *Thaxterogaster* to *Cortinarius* as well as of *Descomyces* and *Setchelliogaster* to *Descolea* were proposed based on the spore morphology and other morphological characters (Bougher and Castellano, 1993). Our molecular data confirm these results, thus supporting the utility of spore morphology for delimiting groups. However, morphological methods have their limits: sometimes the morphology of “Gasteromycetes” is reminiscent of one of the groups from which they have descended, and in other cases, they have been subject to a unique evolution in response to challenge by particular ecological requirements (Reijnders, 2000). When the latter occurs, evolutionary relationships can probably only be elucidated by supplementing the available morphological and ecological data with molecular data.

Conclusions—Within the *Cortinariaceae*, ITS data appear well suited for elucidating relationships around the species level, as sequences of the same taxon from different geographical origins match (Fig. 5). Furthermore, the phylogenetic relationships of sequestrate taxa like *Thaxterogaster*, *Protoglossum*, *Quadrifera*, *Setchelliogaster*, *Descomyces*, and *Hymenogaster* can be determined in relation to their gilled relatives. These results clearly demonstrate that sequestrate taxa evolved independently many times. In most of the cases we studied, emergent, secotioid, and gastroid forms evolved independently from each other, resulting in what appear to be morphologically stable taxa with a tendency to radiate.

However, considerable work is needed not only to determine the phylogenetic relationships of sequestrate fungi, but also to illuminate the molecular mechanisms of the evolution of such striking morphological modifications as represented by sequestrate fungi. This study and others (Hibbett et al., 1997; Miller et al., 2001) further stress the necessity to reunify the form-group “Gasteromycetes” with the “Hymenomycetes.” Neither group can be regarded as isolated from the other, but acknowledgment of natural, morphologically diverse lineages should be the new paradigm for fungal systematics.

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