A framework for a phylogenetic classification in the genus *Cortinarius* (Basidiomycota, Agaricales) derived from morphological and molecular data

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Abstract: Phylogenetic relationships in the genus *Cortinarius* with emphasis on European species were studied by integrating a molecular phylogenetic analysis of nuclear rDNA sequences with morphological, chemical, and ecological data. Internal transcribed spacers (ITS1 and ITS2, including 5.8S) and the D1–D2 regions of nuclear rDNA of 262 *Cortinarius* species including most of the recognised subgenera from Europe, South America, Australia, Tasmania, New Zealand, and Asia were sequenced and analysed by maximum likelihood and neighbour joining. Our morphological and molecular analyses showed considerable disagreement with earlier morphology-based hypotheses concerning the infrageneric delimitations in *Cortinarius*. We propose a new base for a more natural classification system in *Cortinarius* involving the taxonomic rearrangement of the species into the following major lineages that we informally designate as "clades" at this time: (1) phlegmacioid species are included in *Alluti*, *Amarescentes*, *Arguti*, *Calochroi*, *Caerulescentes*, *Percomes*, *Phlegmacioides*, *Phlegmacium*, *Purpurascentes*, *Scauri*, and *Vulpini*; (2) the *Heterocliti* clade includes species of the subgenera *Leprocybe* and *Cystogenes*; (3) most of the *Myxacium* species are included in the *Delibuti* and *Myxacium* clades; (4) part of *Rozites* are included in the *Rozites* clade; (5) the north-hemispheric species of subgenus *Dermocybe* are included in the *Dermocybe* clade and part of the south-hemispheric species in the *Splendidi* clade; (6) part of *Sericeocybe* are integrated in the *Anomali* and *Telamonia* clades; (7) members of subgenus *Cortinarius* are assigned to the *Cortinarius* clade; (8) telamonioid taxa are included in the *Obtusi*, *Renidentes*, and *Telamonia* clades. Several *Cortinarius* species with peculiar patterns of morphology and basidiome coloration had isolated positions in the sequence analysis, and the evolutionary relationships among the major lineages remained unresolved. Our results suggest the convergent evolution of certain morphological traits across phylogenetically divergent lineages in *Cortinarius*. However, combinations of features such as pileipellis anatomy, spore shape, veil coloration, basidiome pigments, and stipe shape appear useful to circumscribe clades. Our molecular phylogenetic analysis allows insights in global geographical patterns of species distribution within the major lineages in the genus *Cortinarius*. The clade structure proposed here could be used as backbone for further more detailed systematic studies.

Key words: biogeography, *Cortinarius* phylogeny, ITS, MetaPIGA, molecular phylogenetic analysis, morphological convergence, nrLSU.

Résumé : Les auteurs ont étudié les relations phylogénétiques du genre *Cortinarius*, avec emphase sur les espèces européennes, en intégrant une analyse phylogénétique moléculaire de séquences du rADN, avec des données morphologiques, chimiques et écologiques. Ils ont séquencé des espaceurs internes transcrits (ITS1 et ITS2, incluant le 5,8S) et les régions D1–D2 de l'ADN nucléique, chez 262 espèces de *Cortinarius*, incluant la plupart des sous-genres de l'Europe, de l'Amérique du Sud, de l'Australie, de la Nouvelle-Zélande et de l'Asie, et il ont analysé la parenté maximum probable et les liens entre voisins. Ces analyses morphologiques et moléculaires montrent des écarts considérables avec les hypothèses antécédentes, basées sur la morphologie, concernant les délimitations chez les *Cortinarius*. Les auteurs proposent une nouvelle base pour établir un système de classification plus naturel des *Cortinarius*, impliquant le réarrangement taxonomique des espèces, au sein des lignées principales suivantes, qu'ils nomment « clades » pour le moment : (1) les espèces phlegmacioÿdes sont incluses dans les *Alluti*, *Amarescentes*, *Arguti*, *Calochroi*, *Caerulescentes*, *Percomes*, *Phlemacioides*, *Phlegmacium*, *Purpurascentes*, *Scauri* et *Vulpini*; (2) le clade *Heterocliti* inclut les espèces des sous-genres *Leprocybe* et *Cystogenes*; (3) on inclut la plupart des espèces de *Myxacium* dans les clades *Delibuti* et *Myxacium*; (4) une partie des espèces de *Rozites* est incluse dans le clade *Rozites*; (5) les espèces de l'hémisphére nord du genre *Dermocybe* sont placées dans le clade *Dermocybe*, et une partie des espéces de l'hémisphère sud, dans le clade *Splendidi*; (6) une partie des *Seroceocybe* sont intégrés aux clades *Anomali* et *Telamonia*; (7) on assigne les membres du sous genre *Cortinarius*, au clade *Cortinarius*; (8) les taxons télamonioÿdes se retrouvent dans les clades *Obtusi*, *Renidentes* et *Telamonia*. Plusieurs espèces de *Cortinarius*, possédant des patrons morphologiques et des coloris particuliers, occupent des positions isolées dans les analyses de séquences, et les relations évolutives entre les lignées principales demeurent irrésolues. Les résultats suggèrent

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une évolution convergente de certains traits morphologique, pour l'ensemble des lignées phylogénétiques des *Cortinarius*. Cependant, les combinaisons de caractères, comme l'anatomie des pileipellis, la forme des spores, la coloration du voile, les pigments des basidiomes et la forme des stipes, semblent utiles pour circonscrire les clades. L'analyse phylogénétique moléculaire effectuée, ouvre les horizons sur les patrons géographiques globaux de la distribution des espèces, parmi les lignées majeures du genre *Cortinarius*. La structure des clades, proposée par les auteurs, pourrait être utilisée comme base pour des analyses systématiques plus poussées.

Mots clés : biogéographie, phylogénie des *Cortinarius*, ITS, MetaPIGA, analyse phylogénétique moléculaire, convergence morphologique, nrLSU.

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Introduction

Cortinarius Fr. is one of the taxonomically most complex genera in the basidiomycetes, with ca. 2000 species described so far. Morphologically, members of this genus are distinguished from other genera of Agaricales by brown and ornamented basidiospores and usually by a fugacious veil forming a fine cobweb (cortina) between stem and cap edge. The basidiomes of *Cortinarius* species exhibit a striking variety of forms and colours. *Cortinarius* species occur in many distinct habitats in ectomycorrhizal association with shrubs (*Dryas*) and various trees belonging to the Pinaceae, Fagales, Dipterocarpaceae, Myrtaceae, Rhamnaceae, and Salicaceae. The distribution of many *Cortinarius* species appears to be restricted by tree host distribution or local climatic or edaphic factors, which provide the background for a high degree of regional endemism. At the other end of the spectrum, some species seem to have a wide geographic distribution, being found in Europe and North America (Moser et al. 1994, 1995; Moser and Ammirati 1996, 1997, 1999) or in South America and Australia (Horak and Wood 1990); this reflects the putative influence of past geological events in the present species distribution.

Since Fries (1836–1838) introduced the genus *Cortinarius*, numerous mycologists have contributed to its systematics, describing new species or elaborating regional monographs (e.g., Fries 1878–1884; Ricken 1915; Moser 1960, 1969–1970; Moser and Horak 1975; Brandrud et al. 1990–1998; Horak and Wood 1990; Moënne-Loccoz et al. 1990–2004). In the southern hemisphere, *Cortinarius* species have been relatively little studied. Moser and Horak (1975) monographed most of the species described for South America, recognising the subgenera *Myxacium*, *Telamonia*, *Leprocybe*, *Phlegmacium*, *Dermocybe*, *Icterinula*, *Sericeocybe*, *Cystogenes*, and *Paramyxacium.* Subsequently, Horak (1988), Horak and Wood (1990), Soop (1999, 2001), and Gasparini (2005) described members of the subgenera *Cortinarius*, *Dermocybe*, *Myxacium*, *Phlegmacium*, *Paramyxacium*, and *Telamonia* from Australia, New Zealand, Tasmania, New Caledonia, and Papua New Guinea. In contrast to that, in the northern hemisphere, especially in Europe, extensive systematic studies have been conducted in the genus *Cortinarius*. Moser (1983) recognised the subgenera *Cortinarius*, *Leprocybe*, *Myxacium*, *Phlegmacium*, *Sericeocybe*, and *Telamonia*, regarding *Dermocybe* as a separate genus. Moënne-Loccoz et al. (1990–2004) and Bidaud et al. (1994) divided *Cortinarius* into the subgenera *Cortinarius*, *Dermocybe*, *Hydrocybe*, *Myxacium*, *Phlegmacium*, and *Telamonia;* Brandrud et al. (1990–1998) recognised the subge-

nera *Cortinarius*, *Dermocybe*, *Myxacium*, *Phlegmacium*, and *Telamonia*. An extensive worldwide taxonomic treatment of the genus *Cortinarius* was published by Moser (1986), who recognised the subgenera *Cortinarius*, *Cystogenes*, *Leprocybe*, *Myxacium*, *Paramyxacium*, *Phlegmacium*, and *Telamonia*. Several of Moser's infrageneric groups contain species spanning different continents; on other hand, the subgenus *Paramyxacium* comprises taxa exclusively restricted to habitats in the southern hemisphere. Some authors recognised *Phlegmacium*, *Dermocybe*, and *Rozites* as separate genera (e.g., Fayod 1889; Earle 1909; Moser 1960, 1986). In all these classification systems, macroscopic features referring to the consistency of both pileus and stipe surface, as well as the coloration of the basidiomes were considered as crucial characters in the circumscription of the major divisions in *Cortinarius*.

In recent years, several attempts have been made to increase the number of traits used for taxonomic purposes in the genus *Cortinarius*. For example, analyses of the pigment content of the basidiomes seem to favour some natural groups in *Cortinarius* (Gruber 1970; Keller 1982; Keller and Ammirati 1983, 1995; Oertel 1984; Steglich and Oertel 1985; Arnold et al. 1987; Gill and Steglich 1987; Keller et al. 1988; Arnold 1993). On the other hand, the relatively few detailed morphological or anatomical studies carried out in *Cortinarius* (Brandrud 1996a, 1996b, 1998a, 1998b; Garnica et al. 2003a, 2003b) suggest that certain microscopic characters are highly significant to circumscribe some natural groups. However, it is necessary to expand both chemical and morphological or anatomical analyses to a number of *Cortinarius* species. Most recently, molecular data have been used to estimate phylogenetic relationships in *Cortinarius* (Liu et al. 1997; Chambers et al. 1999; Høiland and Holst-Jensen 2000; Seidl 2000; Peintner et al. 2001, 2004; Moser and Peintner 2002a, 2002b; Garnica et al. 2003a, 2003b). Molecular phylogenetic analyses have demonstrated that the sequestrate genera *Hymenogaster* p.p., *Quadrispora*, *Protoglossum*, and *Thaxterogaster*, and the agaricoid genera *Rozites*, *Cuphocybe*, and *Rapacea* cluster within *Cortinarius* (Peintner et al. 2001, 2002). Molecular studies have shown the monophyly of the genus *Cortinarius* (including the taxa mentioned before) and that the ITS region of the nuclear rDNA is species specific. These ITS analyses, however, do not support the higher groups recognised in current taxonomic concepts. These molecular studies consistently suggest that characters thought to be of phylogenetic significance in the past have evolved or been lost more than once during the evolution of the genus.

In this study, we inferred estimates of the phylogenetic relationships in the genus *Cortinarius* by sequencing and analysing the ITS (including the gene coding for the 5.8S ribosomal subunit) and also the D1–D2 regions of nuclear rDNA coding for the large ribosomal subunit. The collections sequenced for this study, which represent a wide taxonomic and geographical spectrum of species, were also thoroughly examined macro- and micro-scopically. We compare our molecular phylogenetic hypotheses with the distribution of macro- and micro-scopical characters and also with chemical traits taken from literature. Also we evaluated the infraspecific variation of morphological, chemical, and molecular characters. As a result of these analyses, we propose a new, presumably more natural classification system, splitting the genus *Cortinarius* into several major clades.

This work is still focused on European species, the area for which a representative taxon sampling was possible. We have attempted, however, to explore the global phylogenetic relationships by including a number of *Cortinarius* species from the southern hemisphere. Formal taxonomic consequences and detailed descriptions of the new taxonomic arrangements will be presented in future papers. This study is part of an ongoing project to redefine infrageneric circumscriptions in the genus *Cortinarius* at a worldwide scope.

Materials and methods

Taxon sampling

Cortinarius collections including representative species of most of the subgenera from Europe, Asia, Australia, New Zealand, Tasmania, and South America were used for this study. Information on provenance of the specimens and GenBank accession numbers (National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov) of the DNA sequences used are given in Table 1. For many species, we sequenced several collections from different geographical origins. Initially, alignments of all sequences obtained were analysed by neighbour-joining (Saitou and Nei 1987) in PAUP* (Swofford 2002), followed by successive pruning of sequences to obtain a manageable size of the dataset. In most cases, one sequence per species was considered sufficient for the phylogenetic analyses in the final sampling design. Additionally, *Cortinarius* sequences published previously by us, with GenBank accession No. AF539706–AF539737 (Garnica et al. 2003*a*) and AY174779–AY174864 (Garnica et al. 2003*b*), were used in the phylogenetic analyses.

DNA extraction, amplification, and sequencing

Genomic DNA was isolated from dried herbarium basidiomes using the Qiagen DNeasy Plant Isolation Kit (QIAGEN, Hilden, Germany). In most cases, PCR amplifications of the ITS region (ITS1 and ITS2), 5.8S and D1–D2 domains of the rDNA were performed using the primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′, Gardes and Bruns 1993) / NL4 (5′-GGTCCGTGTTTCAAGACGG-3′, O'Donnell 1993) with concentrations of PCR components and cycling parameters as indicated in Weiß et al. (1998). Alternative primer combinations used for PCR amplifications are described in Garnica et al. (2003*b*). PCR products were purified using the QIAquick kit (QIAGEN),

following the manufacturer's instructions, and the double-stranded DNA was sequenced using the ABI PRISM Big-Dye cycle sequencing kit (Applied Biosystems, Foster City, California) and primers indicated in Garnica et al. (2003*b*). The sequences were produced with automated sequencers ABI 373A or ABI 3100 (Applied Biosystems, Foster City, California).

Phylogenetic analyses

Forward and reverse sequences were assembled and edited using Sequencher, version 4.1 (Gene Codes, Ann Arbor, Michigan). A multiple sequence alignment of 264 *Cortinarius* sequences and *Laccaria amethystina* as outgroup sequence was generated using Clustal X (Thompson et al. 1997), followed by manual adjustments in Se-Al (Rambaut 1996). The final alignment, which has been deposited in TreeBASE (http://www.treebase.org), was 1606 nucleotides long (including gaps), with 653 constant sites. A total of 381 nucleotide positions in regions too divergent to align were excluded from the analyses.

To estimate the phylogenetic relationships from the sequence alignment, we applied a heuristic maximum-likelihood (ML) approach using a "metapopulation" genetic algorithm as implemented in the computer program Meta-PIGA, version 1.0.2b (Lemmon and Milinkovitch 2002), employing the HKY85 model of DNA substitution (Hasegawa et al. 1985). The latter is the richest model available in both MetaPIGA and PAUP* (Swofford 2002; used for the BIONJ analysis described below). To obtain estimates of a maximum-likelihood tree, this method simulates a parallel evolution of several populations of phylogenetic trees during which the trees are subjected to mutation events with subsequent selection in each generation. In this process the populations are linked by the "consensus-pruning" principle: those parts of the trees that are conserved to a high degree across the current populations have lower mutation probabilities. We used four populations, each consisting of four trees, with starting trees obtained by "noisy neighbour-joining" (NNJ), a procedure in which initial trees are built according to the neighbour-joining method (Saitou and Nei 1987) but distorted by stochastical joining ($p = 0.5$) of non-optimal nodes. The mutation process was ruled by the "probability group consensus" criterion; selection was simulated with the "improve selection" option that allows only trees better than the best trees from the previous generations to produce an offspring. Optimization of the transition/transversion rate was enabled and branch length optimization was disabled during the process. The process was stopped by the program (i.e., when the current trees could not be improved by the allowed mutations), and the current best tree of each of the four populations was saved. This metapopulation maximum-likelihood analysis was run independently 100 times on a Macintosh G4 computer, resulting in 400 stored trees.

We determined the tree with the highest likelihood among these 400 trees using a PERL script written by C. Oertel (Bioinformatics Department, Tübingen University, Tübingen, Germany), which is available from M. Weiß. We also computed majority-rule consensus values from the 400 stored trees in PAUP* (Swofford 2002). Since the starting trees of the 100 independent runs of the metapopulation

Table 1. List of *Cortinarius* species used for morphological and molecular analyses in this study.

Taxon	Locality	Voucher No.	GenBank accession No.
Subgenus *Dermocybe*			
C. canarius (E. Horak) Gasparini	Tasmania	HO A20511C4	AY669630
C. globuliformis N. L. Bougher	Australia	PERTH 05506719	AY669602
C. kula Grgurinovic	Tasmania	HO 980515A0	AY669643
C. malicorius Fr.	Austria	TUB O11819	AY669583
C. luteistriatulus (M. M. Moser & E. Horak) E. Valenz. & G. Moreno	Chile	AH 15465	AF539707[b]
C. sanguineus (Wulfen) Fr.	Germany	TUB 011822	AY669582
C. splendidus Peck	Australia	PERTH 05506808	AY669598
C. tubarius Ammirati & A. H. Smith	Germany	TUB 011821	AY669581
C. uliginosus (Berk.) M. M. Moser	Germany	TUB 011823	AY669584
C. walkeri Cooke & Massee	Tasmania	HO A20528A0	AY669632
Subgenus *Icterinula*			
C. amoenus (M. M. Moser & E. Horak) E. Valenz. & G. Moreno	Chile	TUB O11467	AF5397211[b]
C. flavifucatus (E. Horak & M. M. Moser) E. Valenz. & G. Moreno	Chile	TUB 011476	AF539709[b]
C. icterinus (E. Horak) E. Horak	Chile	TUB 011477	AF539720[b]
C. olivaceobubalinus (M. M. Moser) Garnica	Chile	TUB 011483	AF539736[b]
C. olivaceofuscus Kühner	Germany	TUB 011820	AY669585
C. obscurooliveus (M. M. Moser) Garnica	Chile	TUB 011482	AF539708[b]
Subgenus *Cortinarius*			
C. hercynicus (Pers.) Brandr.[a]	Germany	TUB O11824	AY669580
C. violaceus (L.) Fr.[a]	Germany	TUB 011825	AY669579
C. violaceus (L.) Fr.	Australia	PERTH 05506794	AY669578
Subgenus *Cystogenes*			
C. austrolimonius var. *ochrovelatus* M. M. Moser	Chile	TUB 011468	AF539706[b]
Subgenus *Leprocybe*			
C. bolaris (Pers.: Fr.) Fr.	Germany	TUB 011524	AY669596
C. cotoneus Fr.[a]	Germany	TUB 011826	AY669597
C. callisteus (Fr.) Fr.[a]	Germany	TUB 011827	AY669594
C. rubellus Cooke	Germany	TUB 011828	AY669595
C. rubicundulus (Rea) A. Pearson	Germany	TUB 011829	AY669599
C. saniosus (Fr.: Fr) Fr.	Germany	TUB 011830	AY669621
Subgenus *Myxacium*			
C. archeri Berk.[a]	Australia	PERTH 05506395	AY669610
C. collinitus (Pers.) Fr.	Germany	TUB 011832	AY669588
C. croceocoeruleus (Pers.: Fr.) Fr.	Germany	TUB 011833	AY669590
C. delibutus Fr.	Germany	TUB 011834	AY669587
C. erythraeus Berk.	Australia	PERTH 05506727	AY669605
C. lustrabilis Moënne-Loccoz	Germany	TUB 011835	AY669586
C. magellanicus Speg.[a]	Chile	TUB 011480	AF539719[b]
C. malvaceus E. Horak	Chile	TUB 011836	AY669611
C. mucosus (Bull.: Fr.) Fr.	Germany	TUB 011837	AY669591
C. quaresimalis Gasparini	Tasmania	HO A20606A5	AY669616
C. rotundisporus Berk.	Australia	PERTH 05255074	AY669612
C. sinapicolor J. B. Cleland[a]	Australia	PERTH 05506778	AY669604
C. salor Fr.	Germany	TUB 011838	AY669592
C. stillatitius Fr.	Germany	TUB 011587	AY669589
C. submagellanicus Gasparini[a]	Tasmania	HO A20518A1	AY669614
C. trivialis Lge.	Germany	TUB 011839	AY669593
Subgenus *Phlegmacium*			
Cortinarius sp.	New Zealand	PDD 77486	AY669644
C. acidophilus Brandr.	Norway	O-125826	AY669524
C. alboaggregatus Soop[a]	New Zealand	PDD 77472	AY669620
C. aleuriosmus Maire[a]	Germany	TUB 011840	AY669537
C. anserinus (Velen.) R. Hry.[a]	Germany	TUB 011404	AY174806[c]
C. arcuatorum R. Hry.[a]	Germany	TUB 011403	AY174824[c]
C. argutus Fr.	Norway	O-60164	AY669535
C. atrovirens Kalchbr.[a]	Germany	TUB 012145	AY174848[c]
C. allutus Fr.	Germany	TUB 011864	AY669532
C. aurantiorufus Garnica	Chile	TUB 011291	AF539710[b]
C. aureocalceolatus M. M. Moser & Peintner	Germany	TUB 011842	AY669569

Table 1 (*continued*).

Taxon	Locality	Voucher No.	GenBank accession No.
C. aureofulvus M. M. Moser	Germany	TUB 011831	AY669571
C. aureopulverulentus M. M. Moser	Germany	TUB 011843	AY669568
C. australis Gasparini	Tasmania	HO A20420A0	AY669515
C. austrocyanites Soop	New Zealand	PDD 70498	AY669626
C. austroturmalis M. M. Moser & E. Horak	Chile	TUB 011469	AF539730[b]
C. austrosaginus Gasparini	Tasmania	HO 980509A0	AY669619
C. austrovaginatus Gasparini	Tasmania	HO 990125A1	AY669635
C. balteatoalbus var. *areni-silvae* Brandr.	Norway	O-125960	AY669533
C. balteatoalbus var. *balteatoalbus* R. Hry.	Switzerland	O-63269	AY669517
C. balteatocumatilis R. Hry. ex P.D. Orton	Germany	TUB 011440	AY174801[c]
C. balteatus Fr.	Germany	TUB 011844	AY669526[c]
C. borgsjoensis Brandr.	Sweden	O-65492	AY669567
C. boudieri R. Hry. ex R. Hry.[a]	Germany	TUB 011402	AY174860[c]
C. caelicolor E. Horak & M. M. Moser	Chile	TUB 011470	AF539715[b]
C. caeruleoeburneus Gasparini	Tasmania	HO 990311A4	AY669634
C. caerulescens (Schaeff.) Fr.	Germany	TUB 012146	AY174863[c]
C. caerulescentium R. Hry.	Germany	TUB 011846	AY669515
C. caesiocortinatus Schaeff.[a]	Germany	TUB 011400	AY174809[c]
C. caesiostramineus R. Hry.[a]	Germany	TUB 011845	AY669519
C. caesiocanescens M. M. Moser	Germany	TUB 011847	AY669546
C. caligatus Malençon	Italy	TEB 153–84	AY669553
C. calochrous Fr.[a]	Germany	TUB 011398	AY174838[c]
C. calochrous var. *coniferarum* (M. M. Moser) Quadr.[a]	Germany	TUB 011385	AY174842[c]
C. camptoros Brandr. & Melot	Germany	TUB 011848	AY669540
C. caroviolaceus P. D. Orton[a]	Germany	TUB 011849	AY669559
C. catharinae Cons.	Germany	TUB 011850	AY669560
C. cedretorum Maire	Germany	TUB 011851	AY669564
C. cephalixus Fr.[a]	Germany	TUB 011395	AY174783[c]
C. cereifolius (M. M. Moser) M. M Moser[a]	Germany	TUB 011426	AY174847[c]
C. cervinus M. M. Moser & E. Horak	Chile	TUB 011472	AF539711[b]
C. citrinolilacinus (M. M. Moser) M. M. Moser	Germany	TUB 011442	AY174830[c]
C. citrinus J.E. Lange ex P.D. Orton[a]	Germany	TUB 012147	AY174820[c]
C. claricolor Fr.[a]	Germany	TUB 011852	AY669522
C. claroflavus R. Hry.[a]	Germany	TUB 011427	AY174852[c]
C. cliduchus Fr.	Germany	TUB 011860	AY669558
C. chalybaeus Soop	New Zealand	PDD 77482	AY669613
C. coalescens Kärcher & Seibt[a]	Norway	O-125961	AY669552
C. coelopus Gasparini	Tasmania	HO 990504A3	AY669640
C. columbinus M. M. Moser & E. Horak	Chile	TUB 011473	AF539735[b]
C. corrosus Fr.	Germany	TUB 011588	AY669562
C. crassus Fr.	Germany	TUB 011589	AY669544
C. cretax Soop	New Zealand	PDD 73148	AY669622
C. cumatilis Fr.	Germany	TUB 011417	AY174812[c]
C. cupreorufus Brandr.[a]	Austria	TUB 011418	AY174831[c]
C. delaportei R. Hry.[a]	Germany	TUB 011853	AY669534
C. dibaphus Fr.	Germany	TUB 011437	AY174819[c]
C. dionysae R. Hry.	Germany	TUB 011856	AY669523
C. aff. *dionysae* R. Hry.	Germany	TUB 011450	AY174813[c]
C. effundens M. M. Moser & E. Horak	Chile	TUB 011854	AY669601
C. elegantior (Fr.) Fr.[a]	Germany	TUB 011394	AY174851[c]
C. elegantissimus R. Hry.	Germany	TUB 011855	AY669565
C. flammuloides E. Horak & M. M. Moser	Chile	TUB 011475	AF539716[b]
C. flavovirens R. Hry.[a]	Germany	TUB 011454	AY174841[c]
C. fraudulosus Britzelm.	Germany	TUB 011870	AY669551
C. fulmineus (Fr.) Fr. (= *C. alcalinophilus* R. Hry.)	Germany	TUB 011433	AY174837[c]
C. fulvocitrinus Schaeff. ex Brandr.[a]	Germany	TUB 011434	AY174828[c]
C. glaucopus (Schaeff.: Fr.) Fr.[a]	Germany	TUB 011414	AY174787[c]
C. gracilior (M. M. Moser) M. M. Moser[a]	Germany	TUB 011857	AY669525
C. haasii (M. M. Moser) M. M. Moser[a]	Germany	TUB 011858	AY669561
C. infractus (Pers.: Fr.) Fr.[a]	Germany	TUB 011384	AY174779[c]
C. infractus var. *obscurocyaneus* (Secr. ex J. Schröt.) Quadr.	Germany	TUB 011859	AY669536
C. iringa Soop	New Zealand	PDD 73135	AY669624

Table 1 (*continued*).

Taxon	Locality	Voucher No.	GenBank accession No.
C. ionochlorus Maire[a]	Germany	TUB 011430	AY174834[c]
C. lacteus Gasparini	Tasmania	HO A20504A2	AY669642
C. langei R. Hry. ex R. Hry.	Germany	TUB 011861	AY669527
C. largus Fr.	Germany	TUB 011455	AY669542
C. latobalteatus M. M. Moser	Germany	TUB 011862	AY669550
C. lavendulensis Clel.[a]	Australia	PERTH 05506735	AY669617
	Tasmania	HO 990304A2	AY669631
C. lustratus Fr.	Germany	TUB 012148	AY174853[c]
C. magicus Eichh.	Germany	TUB 011863	AY669547
C. mairei (M. M. Moser) M. M. Moser	Austria	IB 93/619	AY669548
C. meinhardii Bon[a]	Germany	TUB 011443	AY174840[c]
C. minoscaurus Soop	New Zealand	PDD 71005	AY669628
C. multiformis Fr.	Germany	TUB 011841	AY669531
C. multiformis Fr. sensu M. M. Moser	Germany	TUB 011410	AY174846[c]
C. mussivus (Fr.) Melot	Germany	TUB 011412	AY174814[c]
C. myxoclaricolor M. M. Moser	Chile	TUB 011481	AF539733[b]
C. nanceiensis Maire[a]	Germany	TUB 011389	AY174855[c]
C. nanceiensis var. *bulbopodius* Chevassut & R. Hry.	Germany	TUB 011865	AY669520
C. nymphaeicolor Reum.	Germany	TUB 011866	AY669566
C. obsoletus Kühner	Germany	TUB 011912	AY669549
C. odoratus (Joguet ex M. M. Moser) M. M. Moser	Germany	TUB 011438	AY174836[c]
C. odorifer Britzelm.	Germany	TUB 011383	AY174817[c]
C. osmophorus P. D. Orton[a]	Germany	TUB 011399	AY174815[c]
C. papulosus Fr.	Germany	TUB 011867	AY669555
C. paracephalixus Bohus	Netherlands	Reinders-87	AY669516
C. patibilis Brandr.	Norway	O-125906	AY669543
C. percomis Fr.	Germany	TUB O11868	AY669529
C. permagnificus E. Horak	Chile	AH 19524	AF539722[b]
C. persicanus Soop[a]	New Zealand	PDD 70507	AY669641
	Tasmania	HO 990304A2	AY669639
C. polymorphus R. Hry.	Germany	TUB 011869	AY669545
C. populinus Brandr.	Norway	O-58647	AY669521
C. porphyropus (Alb. & Schw.) Fr.	Germany	TUB 011451	AY174854[c]
C. praestans (Cordier) Gillet[a]	Germany	TUB 011460	AY174804[c]
C. prasinus Fr. sensu Konr. & Maubl.	Germany	TUB 011431	AY174835[c]
C. provencalis M. M. Moser[a]	Germany	TUB 011439	AY174818[c]
C. pseudoglaucopus (Schaeff. ex M. M. Moser) Quadr.[a]	Germany	TUB 011872	AY669573
C. pseudotriumphans M. M. Moser & E. Horak	Chile	TUB 011873	AY669600
C. pseudovulpinus R. Hry.	Germany	TUB 011874	AY669557
C. pugionipes M. M. Moser	Chile	TUB 011484	AF539713[b]
C. punctatisporus Garnica	Chile	TUB 011290	AF539714[b]
C. purpurascens Fr.	Germany	TUB 011401	AY174858[c]
C. purpurascens var. *largusoides* Cetto	Germany	TUB 011871	AY669538
C. rapaceus var. *luridus* M. M. Moser & E. Horak[a]	Chile	TUB 011485	AF539724[c]
C. rufoolivaceus Fr.[a]	Germany	TUB 011405	AY174845[c]
C. saginus (Fr.: Fr.) Fr.	Germany	TUB 011419	AY174797[c]
C. salmaster Gasparini	Tasmania	HO A20528A3	AY669618
C. saporatus Britzelm.	Germany	TUB 011880	AY669570
C. scaurus Fr.[a]	Germany	TUB 011456	AY174808[c]
C. sclerophyllarum Gasparini	Tasmania	HO A20430A6	AY669637
C. sejunctus Gasparini	Tasmania	HO 990125A0	AY669636
C. serarius Fr.	Norway	O-65724	AY669541
C. sodagnitus R. Hry.	Germany	TUB 011428	AY174829[c]
C. spadicellus (M. M. Moser) G. Garnier	Norway	O-65723	AY669539
C. splendens R. Hry.[a]	Germany	TUB 011411	AY174833[c]
C. stephanopus M. M. Moser & E. Horak	Chile	TUB 011875	AY669603
C. suaveolens Bat. & Joachim	Germany	TUB 011876	AY669574
C. subarquatus (M. M. Moser) M. M. Moser	Germany	TUB 01189	AY669563
C. subtortus (Pers.: Fr.) Fr.[a]	Germany	TUB 011382	AY174857[c]
C. sulphurinus Quél.	Germany	TUB 011908	AY669572
C. talus Fr.	Germany	TUB 011877	AY669530
C. terpsichores Melot[a]	Germany	TUB 011878	AY669554

Table 1 (*continued*).

Taxon	Locality	Voucher No.	GenBank accession No.
C. tiliae Brandr.	Norway	O-63407	AY669556
C. triumphans (Fr.) Fr.[a]	Germany	TUB 011461	AY174799[c]
C. turmalis Fr.[a]	Germany	TUB 011393	AY174782[c]
C. vacciniophilus Brandr.	Norway	O-125949	AY669518
C. vaginatus E. Horak & M. M. Moser	Chile	TUB 011879	AY669609
C. variicolor (Pers.: Fr.) Fr.[a]	Germany	TUB 011416	AY174795[c]
C. variiformis Malençon	Germany	TUB 011409	AY174791[c]
C. varius Fr.[a]	Germany	TUB 011392	AY174792[c]
C. vinaceolamellatus J. B. Cleland	Australia	PERTH 05506786	AY669608
C. violaceomaculatus Brandr.[a]	Sweden	O-125787	AY669528
C. viridocoeruleus Chev. & R. Hry.[a]	Germany	TUB 011408	AY174788[c]
C. vulpinus (Velen.) R. Hry.	Germany	TUB 011406	AY174811[c]
C. xanthophyllus Cooke[a]	Germany	TUB 011457	AY174827[c]
Subgenus *Sericeocybe*			
C. alboviolaceus (Pers.: Fr.) Fr.	Germany	TUB 011882	AY669657
C. anomalus Fr.	Germany	TUB 011883	AY669645
C. caninus Fr.	Germany	TUB 011884	AY669646
C. cyanites Fr.	Germany	TUB 011885	AY669647
C. diosmus Kühner	France	TUB 011886	AY669661
C. malachius (Fr.) Fr.	Germany	TUB 011887	AY669681
C. pholideus (Fr.: Fr.) Fr.[a]	Germany	TUB 011520	AY669694
C. spilomeus (Fr.: Fr.) Fr.	Germany	TUB 011523	AY669654
C. tasmacamphoratus Gasparini	Tasmania	HO A20606A0	AY669633
C. turgidus Fr.	Germany	TUB 01188	AY669689
Subgenus *Telamonia*			
Cortinarius sp.	Germany	TUB 011906	AY669656
C. acutovelatus (Pers.: Fr.) Fr.[a]	Germany	TUB 011513	AY669655
C. alnetorum (Velen.) M. M. Moser	Germany	TUB 01192	AY669695
C. anthracinus (Fr.) Fr.	Germany	TUB 01190	AY669670
C. aprinus Melot	Germany	TUB 01191	AY669663
C. ardesiacus Gasparini	Tasmania	HO 970419A0	AY669650
C. armillatus (Alb. & Schwein.) Fr.[a]	Germany	TUB 01193	AY669671
C. austroduracinus M. M. Moser	Chile	TUB 011522	AY669653
C. balaustinus Fr.	Germany	TUB 011894	AY669693
C. belleri M. M. Moser	Spain	TUB 011895	AY669685
C. biformis Fr.	Germany	TUB 011896	AY669688
C. bivelus (Fr.: Fr.) Fr.	Germany	TUB 011897	AY669682
C. bovinus Fr. ss. M. M. Moser	Germany	TUB 011898	AY669691
C. bulliardii (Pers.) Fr.	Germany	TUB 011899	AY669659
C. cagei Melot	Germany	TUB 011514	AY669676
C. candelaris Fr.[a]	Germany	TUB 011518	AY669675
C. carneolus M. M. Moser & E. Horak	Chile	TUB 011471	AF539712[b]
C. casimiri (Velen.) Huijsman	Germany	TUB 011449	AY669679
C. cinnabarinus Fr.[a]	Germany	TUB 011508	AY669662
C. cystidiocatenatus Gasparini[a]	Tasmania	HO A20518A6	AY669651
C. duracinus Fr.	Germany	TUB 011517	AY669674
C. elaphinus M. M. Moser	Chile	TUB 011474	AF539725[b]
C. erythrinus (Fr.) Fr.	Germany	TUB 011900	AY669690
C. evernius (Fr.: Fr.) Fr.[a]	Germany	TUB 011901	AY669686
C. flexipes var. *flabellus* (Fr.: Fr.) Lindström & Melot	Germany	TUB 011902	AY669678
C. flexipes (Pers.: Fr.) Fr. var. *flexipes*	Germany	TUB 011903	AY669683
C. fulvoconicus M. M. Moser	Chile	TUB 011525	AY669677
C. fulvoiubatus Gasparini	Tasmania	HO 990427A5	AY669649
C. helvelloides (Fr.) Fr.[a]	Germany	TUB 011904	AY669684
C. helveolus Fr.	Germany	TUB 011905	AY669667
C. hemitrichus (Pers.: Fr.) Fr.	Germany	TUB 011509	AY669680
C. hinnuleus Fr.[a]	Germany	TUB 011512	AY669665
C. junghuhnii (Fr.) Fr.	Germany	TUB 011907	AY669660
C. laniger Fr.[a]	Germany	TUB 011521	AY669666
C. lignyotus Horak	Chile	TUB 011478	AF539718[b]
C. lividus M. M. Moser	Chile	TUB 011479	AF539734[b]
C. pachynemeus M. M. Moser	Chile	AH 13475	AF539727[b]

Table 1 (*concluded*).

Taxon	Locality	Voucher No.	GenBank accession No.
C. parahumilis Garnica	Chile	TUB 011293	AF539731[b]
C. parvannulatus Kühner	Germany	TUB 011909	AY669664
C. psammocephalus (Bull.) Fr.	Germany	TUB 011910	AY669672
C. renidens Fr.	Germany	TUB 011516	AY669652
C. rubribasalis M. M. Moser & E. Horak	Chile	TUB 011487	AF539732[b]
C. rubricosus (Fr.) Fr. *sensu* M. M. Moser	Germany	TUB 011911	AY669673
C. rubrivelatus Garnica	Chile	TUB 011292	AF539726[b]
C. solis-occasus Melot	Germany	TUB 011914	AY669696
C. squamiger M. M. Moser	Chile	TUB 011488	AF539729[b]
C. subbalaustinus R. Hry.	Germany	TUB 011915	AY669692
C. tenellus M. M. Moser	Chile	TUB 011489	AF539728[b]
C. tortuosus (Fr.: Fr.) Fr.	Germany	TUB 011916	AY669669
C. torvus (Fr.: Fr.) Fr.	Germany	TUB 011515	AY669668
C. tristis E. Horak	Chile	TUB 011917	AY669648
C. umbilicatus Karsten	Germany	TUB 011922	AY669687
C. umbrinolens P. D. Orton	Germany	TUB 011918	AY669658
C. viridibasalis M. M. Moser	Chile	TUB 011490	AF539717[b]
Cuphocybe			
C. aff. *anuensis* Soop	New Zealand	CO 1018	AY669625
C. gymnocephalus nom. ined.	New Zealand	CO 1334	AY669629
C. elaiochorus Peintner, E. Horak, M. M. Moser & Vilgalys	New Zealand	PDD 77748	AY669627
Protoglossum			
C. luteum Massee	Australia	TUB 011923	AY669606
Rozites			
C. caperatus (Pers.: Fr.) Fr.	Germany	TUB 011913	AY669575
C. emodensis Berk.	China	HKAS 365-41	AY669576
C. similis (E. Horak) Peintner, E. Horak, M. M. Moser & Vilgalys	China	HKAS 26154	AY669577
C. subcastanellus E. Horak, Peintner, M. M. Moser & Vilgalys	New Zealand	PDD 77482	AY669623
C. submeleagris Gasparini	Tasmania	HO 990411A1	AY669638
Thaxterogaster			
C. basipurpureus (N. L. Bougher) Peintner & M. M. Moser	Australia	PERTH 04259629	AY669607

Note: Species are listed following the classification system proposed by Moser (1986) with some modifications. Herbarium acronyms: AH, Alcala de Henares Herbarium; HO, Hobart Herbarium; HKAS, Cryptogamic Herbarium, Kunming Institute of Botany, Academia Sinica; PDD, New Zealand Fungal Herbarium; PERTH, Western Australian Herbarium; O, Oslo Herbarium; TEB, private herbarium of Tor Erik Brandrud; TUB, Herbarium Tubingense; CO, private herbarium of Karl Soop.

[a]Several collections from different geographical origins were sequenced for this species.

[b]Sequence from Garnica et al. (2003*a*).

[c]Sequence from Garnica et al. (2003*b*).

maximum-likelihood analysis described above were built following a stochastical principle, these consensus values are used as a measure of support for the groups obtained.

As a different method of molecular phylogenetic inference, we performed neighbour joining (Saitou and Nei 1987) in the BIONJ variant (Gascuel 1997) with 1000 bootstrap replicates (Felsenstein 1985) as implemented in PAUP* on our final alignment. For this analysis, we used the same DNA substitution model (HKY) as for the heuristic maximum-likelihood analysis described above.

Morphological analyses

Macroscopic traits referring to habit, stipe shape, consistency of pileus and stipe, veil colour, colour reactions with 30% KOH and Lugol, coloration (pileus, lamellae, and stipe) of the basidiomes were documented. Examinations of the microscopic structures focused mainly on pileipellis structure using radial freehand sections and on spore morphology using light and scanning electron microscopy (SEM). Light microscopical studies were done from dried material mounted in 3% KOH solution.

Results and discussion

In the following we discuss our new systematical framework in *Cortinarius*, which integrates our molecular phylogenetic analyses and the results of our comparative morphological studies. We deal with the most significant morphological and chemical diagnostic characters, their degree of interspecific variation, and their distribution over the diverse lineages, as well as the interspecific geographical distributions within the different lineages. We consider groups present in our molecular phylogenetic hypothesis (Fig. 1) that can be circumscribed by a combination of morphological, anatomical or chemical characters. At this stage we excluded groups for which a morphological and (or) chemical circumscription is not yet known. In one case (*Scauri*), we included a group that is represented by only one species in the present study; the monophyly of this group has been shown in other studies (see the discussion below). In the following, we give branch support derived from our molecular phylogenetic analyses as "(a/b)", with the first number representing consensus values derived from

the 100 replicates of the ML metapopulation approach procedure detailed above and the second number representing BIONJ bootstrap percentages from 1000 bootstrap replicates. Support values below 50% are not specified.

Caerulescentes clade (58/<50)

Caerulescentes comprises part of the species classified before in the sections *Caerulescentes*, *Calochroi*, *Phlegmacium*, and *Scauri* (Moser 1986), in the sections *Glaucopodes* and *Caerulescentes* (Brandrud et al. 1990–1998), or in the sections *Caerulescentes*, *Calochroi*, *Glaucopodes*, *Multiformes*, *Patibiles*, and *Phlegmacium* (Möenne-Loccoz et al. 1990–2004). The species contained in this clade are macroscopically characterized by a submarginate to marginate bulb, and microscopically by a *pileipellis duplex* (except for *C. mairei*), with a moderately developed gelatinous layer and ellipsoid to amygdaliform spores (except for *C. anserinus*, *C. camptoros*, *C. dionysae*, *C. gracilior*, and *C. viridocoeruleus* with citriform spores). Within this group, we found interspecific similarities of certain coloration patterns, for example, violet-blue colours are widely distributed in the basidiomes (*C. caerulescens* and *C. caesiocanescens*) or more frequently restricted to the lamellae (*C. aleuriosmus*, *C. anserinus*, *C. caesiostramineus*, *C. camptoros*, *C. dionysae*, and *C. viridocoeruleus*). In most taxa of this clade, the context is white, sometimes with pale blue tinges towards the stipe apex. The molecular analyses showed a close relationship between *C. camptoros* and *C. viridocoeruleus*, *C. caerulescens*, and *C. caesiocanescens*, which agrees with the morphological and coloration similarities between these species (Moser 1960; Brandrud et al. 1990–1998). Our molecular phylogenetic analysis supports a close relationship between *C. boudieri*, *C. caerulescentium* and *C. mairei*, which is congruent with both the morphological and coloration patterns. Although the linkage of these species to the remaining *Caerulescentes* species was not significantly supported in our molecular analysis (Fig. 1), we include them in the *Caerulescentes* clade based on morphological similarities and other molecular evidence (Garnica et al. 2003*b*). Our molecular data did not support *C. boudieri* and *C. caerulescentium* as separate species, which is congruent with the poor morphological differentiation on which those species were erected. Most of the species in this clade are exclusively associated with deciduous trees, but some species, such as *C. camptoros*, *C. caesiostramineus*, *C. dionysae*, and *C. mairei*, are associated with both deciduous and coniferous trees. *Cortinarius caesiocanescens* occurs only with coniferous trees mainly in Europe.

Heterocliti clade (100/85)

This clade includes the European species *C. callisteus* and *C. tophaceus* (the latter not included in our analysis; see Peintner et al. 2004), which are currently classified in the subgenus *Leprocybe* (Moser 1983, 1986), and the South American *C. austrolimoneus* var. *ochrovelatus,* which has been classified in the subgenus *Cystogenes* (Moser and Horak 1975). Similar macroscopical (medium-sized basidiomes and clavate stipe) and microscopical patterns (*pileipellis simplex* with a poorly developed epicutis, subglobose spores and strongly incrusted hyphae) support such relationship.

Phlegmacioides clade (80/<50)

This clade comprises taxa that have been classified within several stirpes in the section *Caerulescentes* by Moser (1960, 1986). Subsequently, Brandrud (1998*a*), based on a wide species sampling and detailed microscopical examinations, included these species in the section *Phlegmacioides*. Our results are congruent with Brandrud's classification system, except for the species *C. balteatoalbus* var. *areni-silvae*, *C. crassus,* and *C. serarius*, which were placed outside this clade. Moreover, the molecular analyses consistently included the southern hemispheric species *C. lavendulensis* in the *Phlegmaciodes* clade, rejecting a close relationship of the latter species with species included in the present study in the clade *Caerulescentes*, which has been suggested by Moser and Horak (1975). Microscopically, the species of the *Phlegmacioides* clade are well characterized by a *pileipellis simplex* with a poorly developed gelatinous layer, by the ellipsoid to amygdaliform (rarely subcitriform) spores, by a cylindric to slightly fusiform stipe and the violet to lilac colours restricted mainly to the pileus margin, the stipe apex or the lamellae. In addition, a distinctive yellow reaction with KOH on the context characterizes most of the species in this clade. Ecologically, the European species are associated with coniferous and deciduous trees; *C. lavendulensis* occurs associated with *Eucalyptus* spp. in Australia and Tasmania.

Percomes clade (70/<50)

This clade contains some European species currently placed in several sections of the subgenus *Phlegmacium* (see Moser 1960, 1986; Brandrud et al. 1990–1998; Moënne-Loccoz et al. 1990–2004). Brandrud (1998*a*) placed *C. serarius* in the section *Phlegmacioides*, mainly due to a certain resemblance of habit and pileus colours with *C. delaporti* and *C. papulosus* and to the similar pileipellis structure with *C. cephalixus*. Moser (1960, 1986) included *C. papulosus* in the section *Triumphantes* together with *C. cephalixus*. Subsequently, Brandrud et al. (1990–1998) incorporated *C. papulosus* into the section *Elastici* suggesting a relationship with *C. cephalixus* based on a similar coloration of the veil. Our molecular analyses suggest a close relationship between *C. serarius* and *C. cephalixus*, whereas *C. papulosus* clusters with the southern hemispheric species *C. coelopus* and *Cortinarius* sp. PDD 77486. Furthermore, the molecular data support a close relationship between *C. mussivus*, *C. nanceiensis* and *C. percomis,* which is reflected in the analyses of pigment contents of the basidiomes (Oertel 1984; Steglich and Oertel 1985; Brandrud 1998*b*). Additionally, the species in this clade share a *pileipellis simplex* with a relatively well-developed epicutis and a gelatinous layer, and predominantly ellipsoid to amygdaliform spores. Nevertheless, this clade represents a group with a wide variation of certain macroscopical features. The stipe shape of some species is cylindrical (*C. cliduchus*, *C. mussivus*, *C. nanceiensis*, *C. percomis*) and bulbous in others (*C. coelopus, C. delaporti, C. nanceiensis* var. *bulbopodius*). Violet blue colours are either restricted to the pileus margin (*C. coelopus* and *C. serarius*) or occur exclusively in the lamellae and stipe apex (*Cortinarius* sp. PDD 77486 and *C. delaportei*), or only in the veil at the stipe basis (*C. nanceiensis*), or are completely lacking (*C. cephalixus*,

C. langei, *C. papulosus* and *C. percomis*). Furthermore, a red-wine reaction with 3% KOH on both pileus and lamellae sections characterizes *C. mussivus*, *C. nanceiensis*, *C. nanceiensis* var. *bulbopodium* and *C. percomis*, whereas in *C. papulosus* this reaction is restringued only to the gelatinous layer. In the species *C. cephalixus*, *C. cliduchus*, *C. mussivus*, *C. nanceiensis*, *C. nanceiensis* var. *bulbopodium* and *C. percomis* the external hyphae on the pileus surface appear olive in 3% KOH.

Vulpini clade (85/<50)

Vulpini contains the European species *C. variiformis*, *C. vulpinus*, and *C. obsoletus*, which mainly occur on calcareous soils associated with both conifereous and deciduous trees. Brandrud (1996a) classified *C. variiformis* in section *Phlegmacium,* subsection *Triumphantes,* and *C. vulpinus* in subsection *Vulpini*, respectively. Species within the *Vulpini* clade are macroscopically characterized by a white context of the basidiomes, a well-developed universal veil and a white clavate to subfusiform stipe. At the microscopical level the most distinctive traits are a *pileipellis duplex* composed of a yellowish-brown pigmented well-differentiated hypocutis and a thin epicutis consisting of hyaline to yellowish hyphae with epiparietal striped to crustulose encrustations and a poorly developed gelatinous layer, and ellipsoid to amygdaliform spores. Brandrud (1996a, b) gives a detailed description of macro- and microscopic features and discusses ecological aspects of the species included in this group.

Phlegmacium clade (100/92)

This clade contains the European species *C. populinus* and *C. saginus*, which have been classified in the section *Phlegmacium*, subsections *Arguti* and *Triumphantes*, respectively (Brandrud 1996a). The species included in this clade are characterized macroscopically by a well-developed universal veil, basidiomes with white context and a clavate stipe, and microscopically by a *pileipellis duplex*, with a scarcely developed gelatinous layer and a brown to red-brown hypocutis, and predominantly ellipsoid spores.

Praestantes clade (100/65)

This clade represents a relatively small *Cortinarius* lineage in the boreo-nemoral areas of Europe associated with deciduous (*C. praestans*) or coniferous (*C. claricolor* and *C. cumatilis*) trees. The species included in this clade are characterized macroscopically by relatively large basidiomes with a well-developed universal veil, a predominantly white context and a clavate and attenuated stipe (at least towards the base), and microscopically by subfusoid to amygdaliform spores and a *pileipellis duplex* with a distinctive hypocutis composed of inflated and yellowish-brown pigmented cells, and a poorly developed epicutis and gelatinous layer. The grouping derived from our molecular analyses is congruent with current classifications (Moser 1960, 1986; Brandrud et al. 1990–1998). A close relationship of the species mentioned above with *C. multiformis* and *C. talus* as suggested by Brandrud et al. (1990–1998) was not supported in our analysis.

Arguti clade (86/<50)

This clade contains part of the European phlegmacioid

species that have been classified by Brandrud (1996a, 1996b) in section *Phlegmacium,* subsection *Arguti* (*C. argutus*, *C. fraudulosus*, *C. paracephalixus*) and subsection *Vulpini* (*C. pseudovulpinus*). Members of the *Arguti* clade are distinguished macroscopically by a white, in older basidiomes ochraceous pileus, a white context, white lamellae, sometimes with a pink tone, a distinct veil, a clavate to attenuate and white stipe, and microscopically by a *pileipellis duplex* with a relatively thin epicutis and gelatinous layer, and amygdaliform spores. A detailed characterization including infra- and interspecific phenotypic variation of the species mentioned above are given by Brandrud (1996a, 1996b). The species included in our concept of section *Arguti* are associated with deciduous trees, except for *C. fraudulosus*, which grows associated with coniferous trees.

Myxacium clade (83/<50)

This clade includes both hypogeous and epigeous species. Moser (1986) classified part of the species that then belonged to subgenus *Myxacium* in two separate sections; *Myxacium* (*C. collinitus*, *C. mucosus*, and *C. trivialis*) and *Defibulati* (*C. stillatitius*). Subsequently, Brandrud et al. (1990–1998) placed the species included here in the *Myxacium* clade in the section *Colliniti*. Our molecular analysis shows two well-supported subgroups; the first contains the European species *C. collinitus*, *C. mucosus*, and *C. trivialis* with a glutinous epicutis and veil, and the second includes the European *C. stilliatitius*, the Australian hypogeous *C. basipurpureus* and two *Cuphocybe* species (*C. gymnocephalus* and *C.* aff. *anuensis*) from New Zealand. The members of the second subclade are distinguished by the lack of clamp connections and a poorly developed veil. Similar coloration patterns of the basidiomes, the relatively large and coarsely ornamented spores and pileipellis anatomy (*pileipellis duplex*) agree with this grouping. Additionally, the species included in "*Myxacium* I" by Peintner et al. (2001) and those assigned to the sections *Myxacium* and *Defibulati* by Seidl (2000) and Peintner et al. (2001, 2004) belong to the *Myxacium* clade as conceived here.

Rozites clade (100/100)

This distinctive lineage includes species with ellipsoid to amygdaliform spores, a *pileipellis duplex* and a context with irregular amyloid areas or incrustations (Clémençon 1971). Species of this clade have formerly been classified in the genus *Rozites* and occur in the boreal-meridional areas of Europe and Asia (*C. caperatus*, *C. emodensis* and *C. similis)*, or in New Zealand (*C. meleagris*; Peintner et al. 2002, 2004). The south hemispheric *Rozites* species *C. submeleagris* and *C. subcastanellus* were placed outside of this clade in distant positions in our molecular phylogenetic analysis. A distinctive character of *Rozites* species is the membranous veil, which seems to be a homoplasic trait in *Cortinarius*, as suggested by Peintner et al. (2002).

Purpurascentes clade (100/98)

This clade comprises a surprising combination of species grouped into two well-supported subclades that occur in separate geographic areas. The first subclade includes the northern hemispheric species *C. porphyropus, C. purpurascens,*

and *C. purpurascens* var. *largusoides*, and the second contains the southern hemispheric species *C. australis*, *C. chalybaeus* and *C. submagellanicus*. Although based on habit this group seems somewhat heterogeneous, there are similarities with respect to basidiome coloration, Lugol's (iodine) reaction on the context (Meixner 1975) and also with respect to colour changes when the basidiomes are bruised (not all taxa tested). In addition, this group is circumscribed micromorphologically by a *pileipellis duplex* and ellipsoid to amygdaliform spores, and macroscopically by a cylindrical stipe (except for *C. purpurascens* with a bulbous stipe). The Australian gastroid *C. campbellae* and *C. fragilis* probably also belong to this clade (Peintner et al. 2001, 2004).

Scauri clade

Although in our molecular analyses this lineage is represented only by *C. scaurus,* there is morphological and molecular evidence that other species not included in the present study are closely related to this species. Moser and Peintner (2002*b*), using ITS sequences, revealed a close relationship between *C. scaurus* and the species *C. herpeticus*, *C. fuligineofolius* and *C. montanus*. The phylogenetic relationships indicated in these authors' analyses agree with the macroscopical, microscopical and chemical similarities of these species. In current classification systems, *C. scaurus* has been considered as sister species to *C. purpurascens* and *C. porphyropus* (Moser 1986; Brandrud et al. 1990–1998); however, our molecular analyses do not support such relationship. Ecologically, some species of the *Scauri* clade appear to be highly specialized to the oligotrophic soils of boreal-meridional moorlands and are often growing between *Sphagnum* species in these areas.

Alluti clade (100/81)

Alluti contains the European species *C. allutus*, *C. balteatoalbus* var. *areni-silvae*, *C. multiformis*, and *C. talus*. A close relationship between *C. allutus*, *C. multiformis* and *C. talus* has been suggested by Moser (1960, 1986), who assigned the latter species to section *Phlegmacium*, while *C. balteatoalbus* var. *areni-silvae* was placed in section *Phlegmacioides* by Brandrud (1998*a*). Species in the *Alluti* clade are macroscopically characterized by whitish to argillaceous lamellae and a predominantly whitish and bulbously rounded stipe, and microscopically by relatively small, ellipsoid to amygdaliform spores and a *pileipellis duplex* (the latter not distinct in *C. balteatoalbus* var. *areni-silvae*).

Pseudotriumphantes clade (93/<50)

This lineage contains southern hemispheric *Cortinarius* species associated with *Nothofagus*. It includes the species *C. alboaggregatus* and *C. iringa* from New Zealand and *C. rapaceus* var. *luridus* and *C. pseudotriumphans* from South America, characterized by relatively pale basidiomes. Our molecular data show a close relationship between *C. iringa* and *C. rapaceus* var. *luridus* (both with a slightly bulbous stipe) and between *C. alboaggregatus* and *C. pseudotriumphans* (both with a fusiform stipe). The members of this clade have a *pileipellis duplex* with a well-differentiated hypocutis, and ellipsoid to amygdaliform spores.

Splendidi clade (99/55)

This group includes epigeous (*C. kula*, *C. sejunctus* and *C. splendidus*) and hypogeous (*C. globuliformis*) species restricted to the southern hemisphere (Australia, Tasmania and probably New Zealand) in association with *Eucalyptus*. Species in this clade are characterized micromorphologically by a more or less distinctive *pileipellis duplex* and wide ellipsoidal to subglobose spores, and chemically by similar wine-red to olive colour reactions with 3% KOH. We hypothesize that the species in this clade, which together with the species of the *Dermocybe* clade as conceived here (see below) were included in subgenus *Dermocybe* by Moser (1986), share similar pigments.

Dermocybe clade (100/80)

This clade contains the species *C. malicorius*, *C. olivaceofuscus*, *C. sanguineus, C. tubarius*, and *C. uliginosus*. This grouping is congruent with earlier molecular phylogenetic analyses (Liu et al. 1997; Peintner et al. 2001) and pigment contents (Keller 1982, Keller and Ammirati 1983, Høiland 1984, Gill and Steglich 1987). Furthermore, our morphological analyses showed that the species within the *Dermocybe* clade share a *pileipellis simplex* with a scarcely developed epicutis, relatively small and ellipsoid spores, and a wine-red reaction with 3% KOH on pileus and lamellae. A close relationship between species of subgenus *Dermocybe* from the northern and the southern hemisphere has been suggested by Moser (1986) on the basis of pigment contents. This hypothesis is consistent with our molecular phylogenetic analysis and also with a previous molecular analysis by Peintner et al. (2004).

Anomali clade (97/<50)

Differing opinions exist in the current taxonomic literature concerning the position of the European species *C. anomalus*, *C. bolaris*, *C. caninus*, and *C. spilomeus*. Moser (1986) included these species in the subgenera *Sericeocybe* and *Leprocybe*, whereas Brandrud et al. (1990–1998) and Moënne-Loccoz et al. (1990–2004) assigned them to subgenus *Telamonia* or subgenus *Phlegmacium*, respectively. The South American *C. tristis* was placed in subgenus *Telamonia* by Moser and Horak (1975). The monophyly of *Anomali* was highly supported in our metapopulation ML analysis, which is congruent both with microscopical traits (predominantly *pileipellis duplex*, subglobose spores), and macroscopical similarities (violet colours, mainly restricted to the lamellae) shared by most of its species. A close relationship between the European species *C. spilomeus* and *C. sclerophyllarum* from Tasmania has also been recently suggested by Gasparini (2006) on the basis of macroscopic similarities.

Amarescentes clade (100/95)

This clade includes *C. infractus* and *C. infractus* var. *obscurocyanus* both of which are associated with deciduous and coniferous trees in Europe. We have sequenced five additional collections of *C. infractus* from different geographical origins with a conspicuous molecular heterogeneity (up to 2.6% divergence in the rDNA regions used in the present study; data not shown). These results support the existence of several, morphologically very similar species as sug-

Fig. 1. Phylogenetic relationships in the genus *Cortinarius* based on ITS sequences (including the gene coding for the 5.8S ribosomal sub-unit) and the D1–D2 region of the nuclear rDNA coding for the large ribosomal subunit. Best tree found in 100 heuristic maximum-likelihood searches using a metapopulation approach based on a genetic algorithm as implemented in MetaPIGA (HKY model of DNA substitution, using four populations of four trees each and starting trees obtained by noisy neighbour joining). Numbers above branches specify the percentage with which the respective bipartion was detected in the 100 independent runs of the metapopulation algorithm; numbers below branches are bootstrap percentages obtained from BIONJ analyses (HKY model of DNA substitution) from 1000 random re-samplings of the original alignment. Percentages below 50% are omitted. The terminal branch of *C. rubicundulus* was scaled to 60% of its original length for graphical reasons. The topology was rooted with *Laccaria amethystina*. For details see the text. A, Asia; Aus, Australia; E, Europe; NZ, New Zealand; SA, South America; TAS, Tasmania.
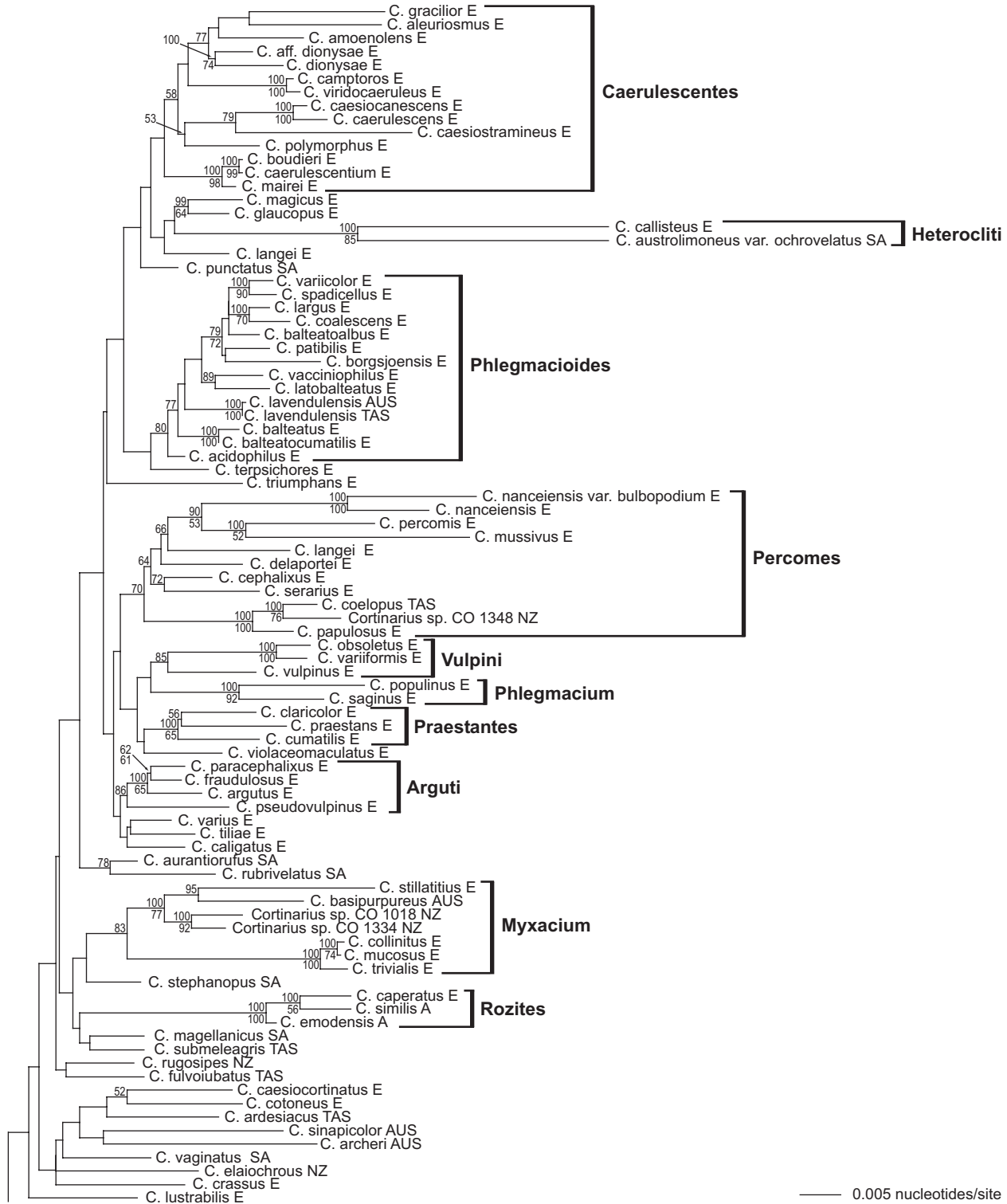
Fig. 1 (continued).
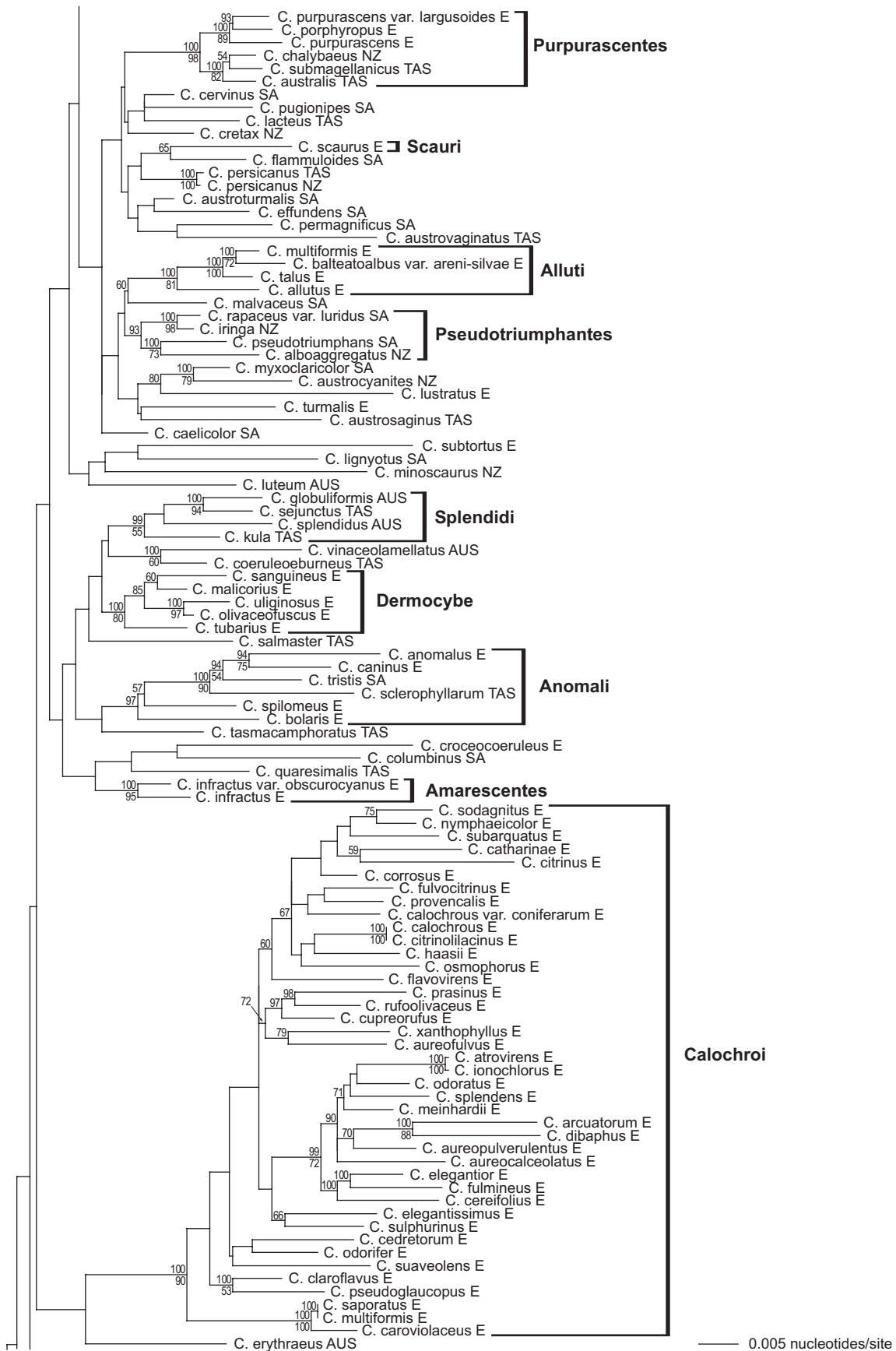
Fig. 1 (concluded).
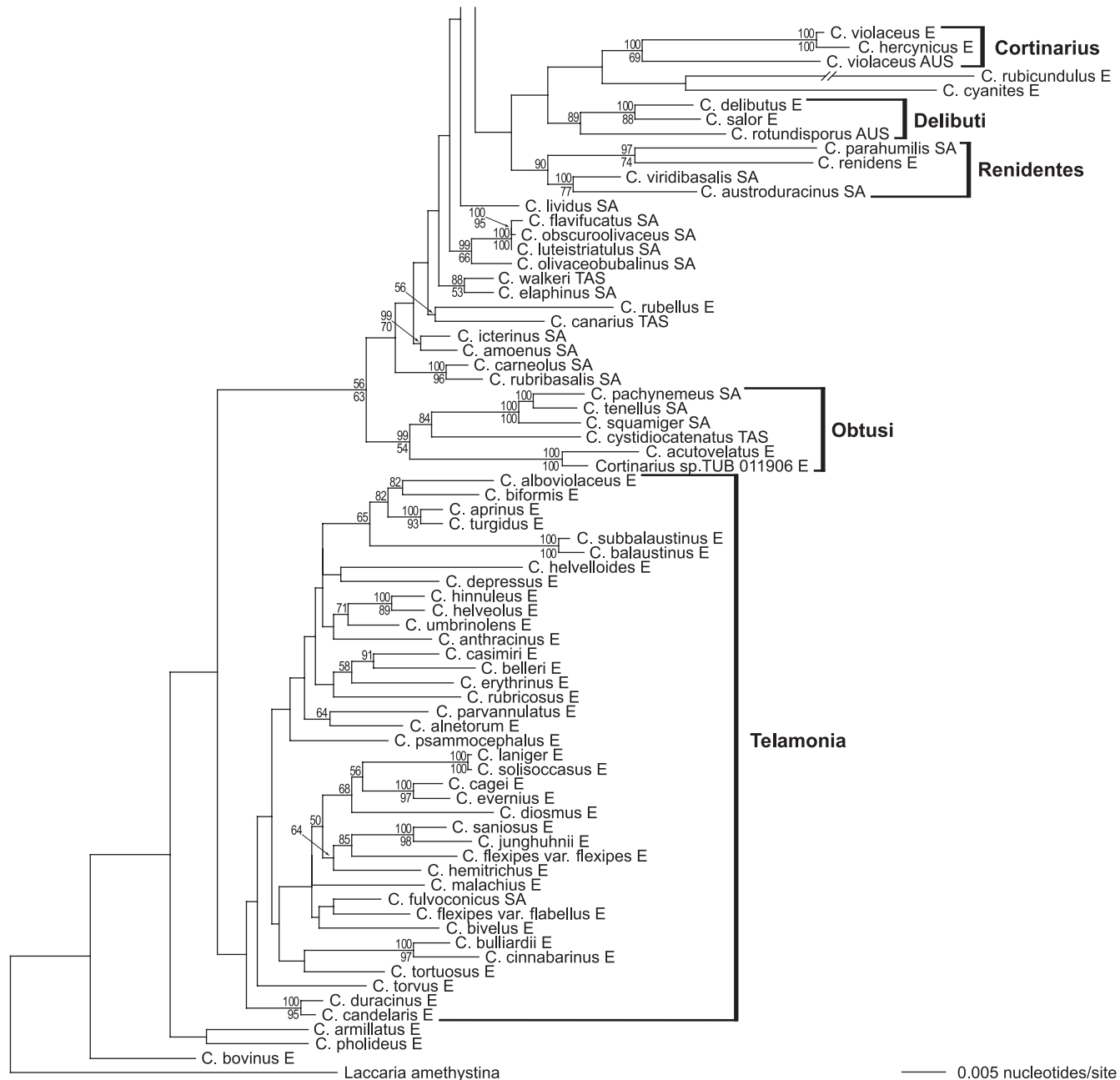
gested by Moser (1960). Both morphological and chemical traits (spore morphology, pileipellis structure; taste, lamellar colour, indole alkaloid content) circumscribe this group well (Moser 1960; Steglich et al. 1984). In a molecular phylogenetic analysis by Peintner et al. (2004), *C. infractus* appeared closely related to the South American *Myxotelamonia* species *C. cinereobrunneus*.

Calochroi clade (100/90)

This clade contains species that have been classified in the sections *Calochroi*, *Scauri* and *Fulvi* (Moser 1960, 1986), in the sections *Multiformes*, *Fulgentes*, and *Laeticolores* (Moënne-Loccoz et al. 1990–2004), or in the sections *Fulvi* and *Calochroi* (Brandrud et al. 1990–1998, mainly based on chemical data). *Calochroi* in our interpretation rep-

resents a typical boreal-meridional element (Europe and North America) with species occurring on calcareous soils, mainly associated with deciduous trees (*Fagus*, *Quercus*) and to a minor degree with coniferous trees (*Picea, Abies*). Macroscopically, all members of this clade are well characterized by a marginate bulb and bright coloured basidiomes, and microscopically by a *pileipellis simplex* (except for *C. aureocalceolatus*) with a well-developed gelatinous layer. Predominantly, they have amygdaliform to citriform and coarsely ornamented spores; in some species the apical part of the spores forms a distinctive papilla. However, owing to the considerable morphological and microscopical/anatomical uniformity within this clade, the interpretation of the internal grouping derived from our molecular data has to be focussed on the pigment contents of the basidiomes. Our

Figs. 2–7. Examples of macro- and micro-scopic convergent traits in the genus *Cortinarius*. Fig. 2. Gastroid habit; *C. basipurpureus* (drawing from dried material). Fig. 3. Marginate bulb at stipe base; *C. minoscaurus*. Fig. 4. Fusiform stipe; *C. alboaggregatus*. Fig. 5. Membranose veil; *C. caperatus*. Fig. 6. *Pileipellis duplex*; *C. alnetorum*. Fig. 7. *Pileipellis simplex*; *C. cedretorum*. Scale bars = 1 cm for Figs. 2–5 and 20 μm for Figs. 6–7.
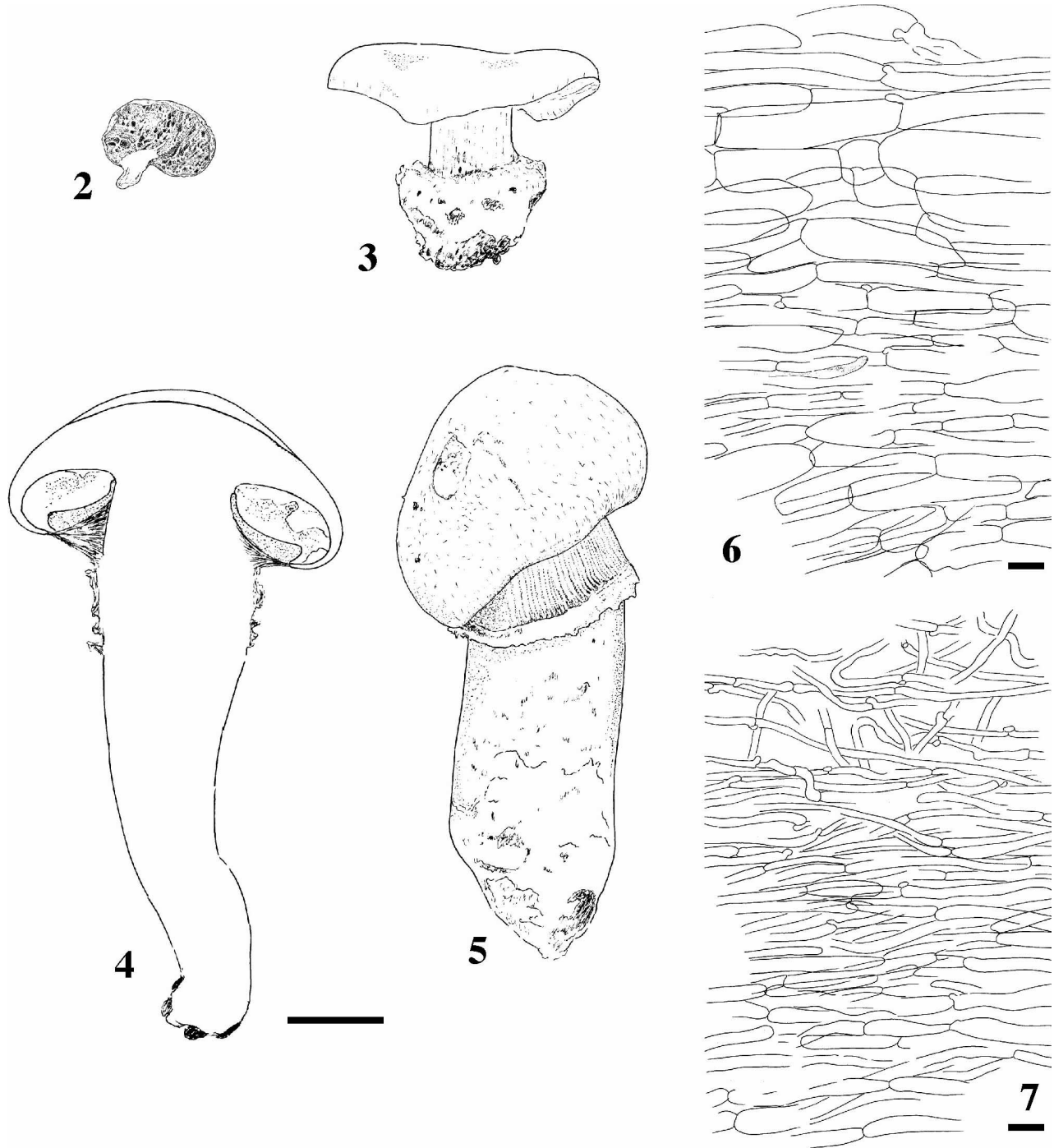
molecular phylogenetic hypothesis is consistent with the division of this lineage into at least five subclades: (1) A weakly supported subclade previously designated as section *Calochroi* by Garnica et al. (2003*b*) contains the taxa *C. calochrous*, *C. calochrous* var. *coniferarum*, *C. citrinolilacinus*, *C. citrinus*, *C. flavovirens,* *C. osmophorus*, and *C. sodagnitus*, and additionally the species *C. catharinae*, *C. corrosus*, *C. haasii*, *C. nymphaeicolor*,

C. provencalis, and *C. subarquatus*. Within the *Calochroi* clade most of the species with violet lamellae, a strongly marginate bulb and an ochraceous-yellow or yellowish green pileus surface belong to this subclade. The gelatinous layer turns vinaceous in 3% KOH in *C. citrinus*, *C. flavovirens*, *C. fulvocitrinus*, and *C. provencalis*, and pink in *C. catherinae*, *C. nymphaeicolor*, and *C. sodagnitus*. (2) A well-supported subclade, largely congruent to subsection

Figs. 8–15. Selected spore morphologies of *Cortinarius* species as seen in SEM. Fig. 8. Amygdaliform with plage; *C. violaceus*. Fig. 9. Subfusoid; *C. effundens*. Fig. 10. Amygdaliform; *C. archeri*. Fig. 11. Citriform; *C. vaginatus*. Fig. 12. Ellipsoid; *C. hemitrichus*. Fig. 13. Subglobose; *C. delibutus*. Fig. 14. Strongly ornamented spores of the hypogeous loculated *C. basipurpureus*. Fig. 15. Strongly ornamented spores of the hypogeous gilled *C. globuliformis*. Scale bars = 4 μm for Figs. 8, 10, 13, and 14; 2 μm for Figs. 9, 11, 12, and 15.

Atrovirentes (Brandrud 1998*b*), includes the taxa *C. arcuatorum*, *C. atrovirens*, *C. aureopulverulentus*, *C. aureocalceolatus*, *C. dibaphus*, *C. meinhardii*, *C. odoratus*, and *C. splendens*. In this subclade we have observed two coloration patterns with KOH mainly of the outermost hyphae of the gelatinous layer. An olive-green reaction is typical for the species *C. atrovirens*, *C. meinhardii*, *C. odoratus*, and *C. splendens,* whereas a pink to vinaceous reaction was observed in the species *C. arcuatorum*, *C. aureocalceolatus*, *C. aureopulverulentus*, and *C. dibaphus*. (3) Another subclade, corresponding to subsection *Elegantiores* (Brandrud 1998*b*), is represented in the present study by the species *C. cereifolius*, *C. elegantior*, and *C. fulmineus*, which have been considered as closely related by Moser (1960). Our phylogenetic analysis supports a close relationship between the subsections *Atrovirentes* and *Elegantiores*, which is congruent with similarities in the pigment contents of the basidiomes (Oertel 1984; Steglich and Oertel 1985; Brandrud 1998*b*). (4) An additional subclade contains the species *C. aureofulvus*, *C. cupreorufus*, *C. prasinus*, *C. rufoolivaceus*, and *C. xanthophyllus*. Members in this subgroup share similar coloration patterns of the basidiomes, KOH reactions, and pigment contents (Oertel 1984; Steglich and Oertel 1985; Brandrud 1998*b*). (5) Our molecular data suggest that *C. multiformis* sensu M.M. Moser (associated with deciduous trees) and *C. saporatus* (associated with coniferous trees) are conspecific; both taxa appear closely related to *C. caroviolaceus*. Phylogenetic results from ITS sequences obtained by Peintner et al. (2001, 2004) also support the monophyly of the *Calochroi* clade and show that this clade additionally includes some sequestrate (*C. bigelowii*, *C. magnivelatus*, *C. verrucisporus*) and non-sequestrate (*C. olympianus, C. flavaurora*) species from North America, which are not included in the present study.

Moser and Horak (1975) and Moser (1986) classified some South American Cortinarii, among them *C. vaginatus,* in section *Fulvi* and *C. stephanopus* in section *Calochroi*. Both taxa were placed outside this clade in isolated positions in our molecular analysis. The European species *C. caesiocortinatus* is similar to some members of section *Calochroi* in coloration and habit, but differs in spore morphology. We sequenced two collections of *C. caesiocortinatus*, one associated with *Fagus sylvatica* and the other with *Picea abies* with somewhat larger spores, which resulted in identical sequences; the species appears separated from the *Calochroi* clade in our molecular phylogenetic tree, in the vicinity of the European *C. cotoneus* and the Tasmanian *C. ardesiacus*, with unclear relationship to the clades here proposed.

Cortinarius clade (100/69)

The European species *C. violaceus* and *C. hercynicus,* together with the Australian specimen designated as *C. violaceus,* but according to our molecular analysis obviously not conspecific with the European species, form a well supported lineage. Molecular phylogenetic analyses published by Peintner et al. (2003, 2004) agree with our results and additionally placed the south-hemispheric *C. atroazulinus* within this clade. A combination of macroscopical and microscopical traits (fleshy basidiomes with dry and tomentose-squamose pileus surfaces; pleuro- and cheilo-cystidia, trichodermal pileipellis, spores with a suprahilar plage and the blood-red colour change with KOH in all parts of the basidiomes) as well as cathechol pigments (Nussbaum et al. 1998) seem to support the monophyly of the type clade of *Cortinarius*. Brandrud et al. (1990–1998) and Moënne-Loccoz et al. (1990–2004) classified *C. violaceus* and *C. hercynicus* within the subgenus *Cortinarius* together with *Leprocybe* and *Dermocybe* species, however, such relationship was not supported by our phylogenetic analysis.

Delibuti clade (89/<50)

This group of myxacioid species comprises *C. delibutus*, *C. rotundisporus*, and *C. salor* characterized by subglobose spores, a *pileipellis duplex* with a gelatinous layer, lilacblue lamellae and a glutinous annular zone in the upper part of the stipe. Additionally, the Australian species *C. australiensis* and the South American species *C. amazonicus* and *C. illitus* seem to belong to this clade (Peintner et al. 2004). *Cortinarius delibutus* and *C. salor* have been considered as closely related taxa, while *C. rotundiporus* has been classified in the subgenus *Phlegmacium* (Moser 1986). Moënne-Loccoz et al. (1990–2004) placed *C. delibutus* and *C. salor* in the subgenus *Phlegmacium*, which is not supported by our data.

Renidentes clade (90/<50)

This telamonioid lineage includes the boreo-nemoral distributed *C. renidens* associated with coniferous trees (Moser et al. 1994) and the South American species *C. austroduracinus*, *C. parahumilis*, and *C. viridibasalis*, which are associated with *Nothofagus* species (Moser and Horak 1979). These species share a similar pileipellis anatomy (*pileipellis duplex*), subglobose basidiospores, a poorly developed to lacking veil, and basidiome coloration. Interestingly, our molecular analysis showed two highly-supported distinctive subclades one of which unites the north hemispheric *C. renidens* with the South American *C. parahumilis.*

Obtusi clade (99/54)

This telamonioid clade includes *C. acutovelatus* and *Cortinarius* sp. TUB 011906 occurring in the boreal-meridional areas in Europe, *C. pachynemeus*, *C. tenellus*, and *C. squamiger* from South America and *C. cystidiocatenatus* from Tasmania, with the European, the South American and
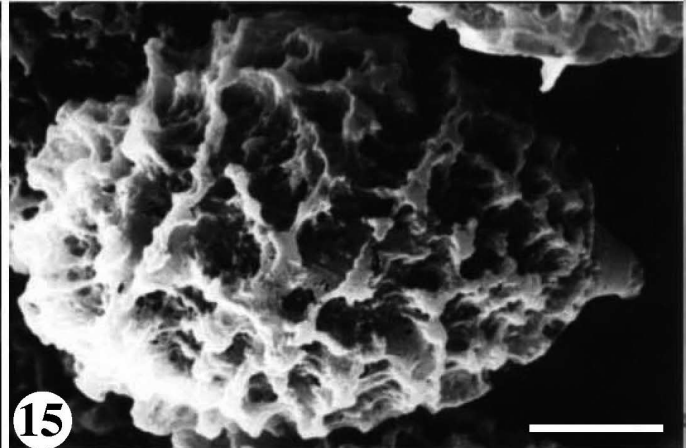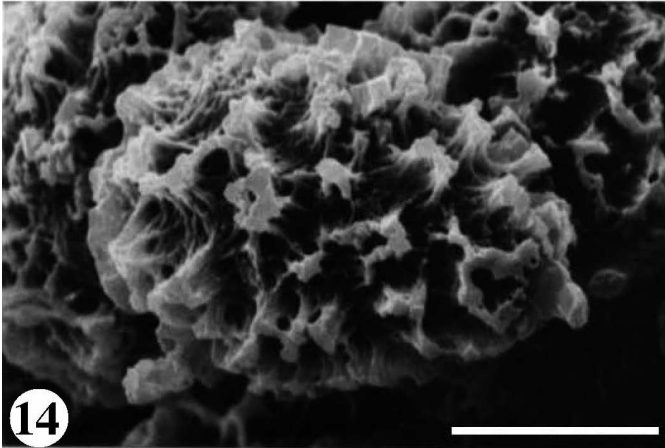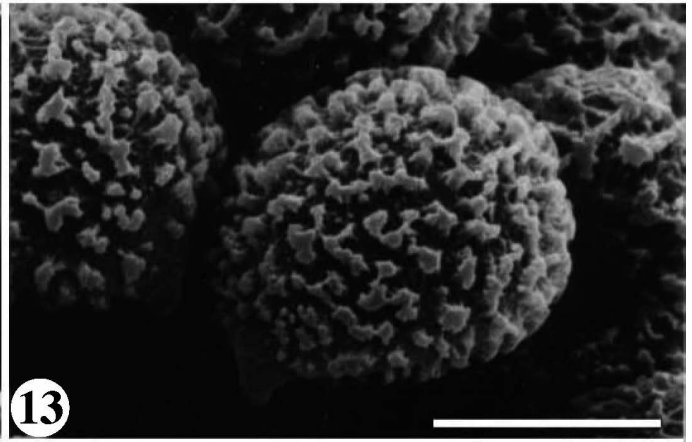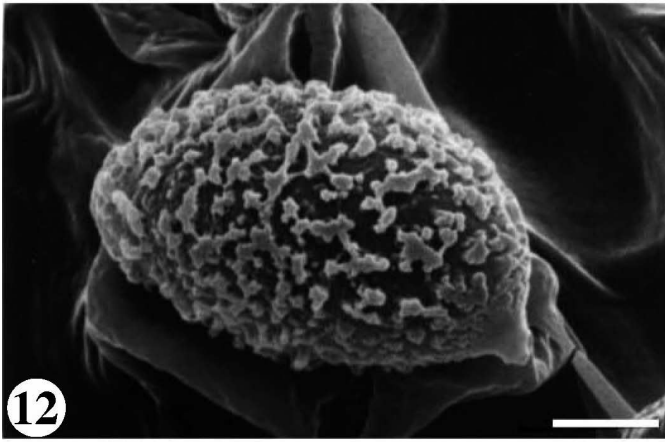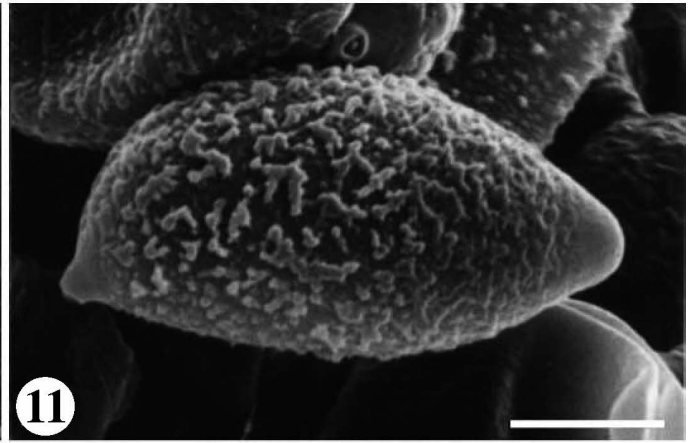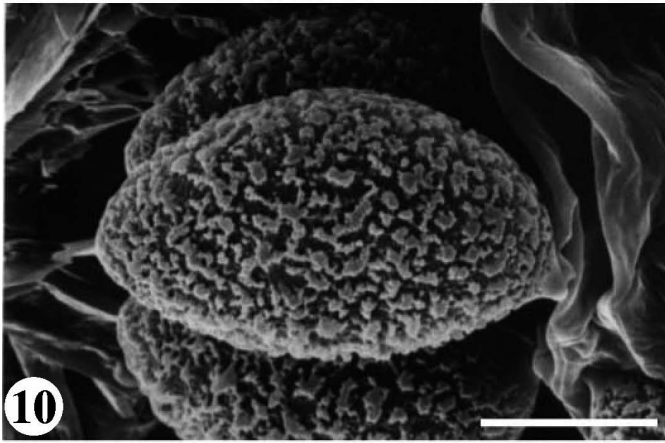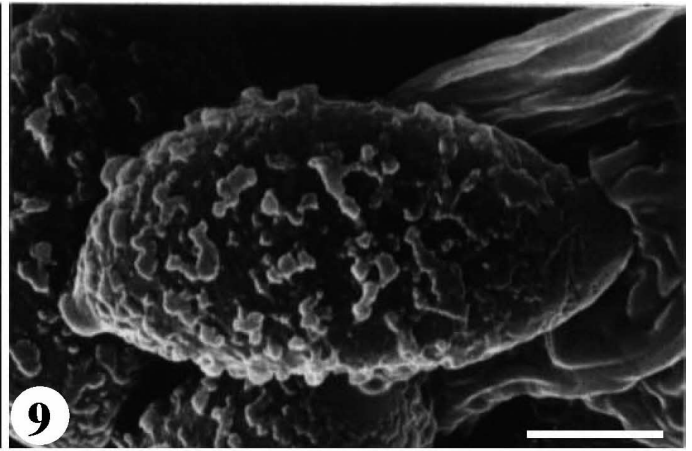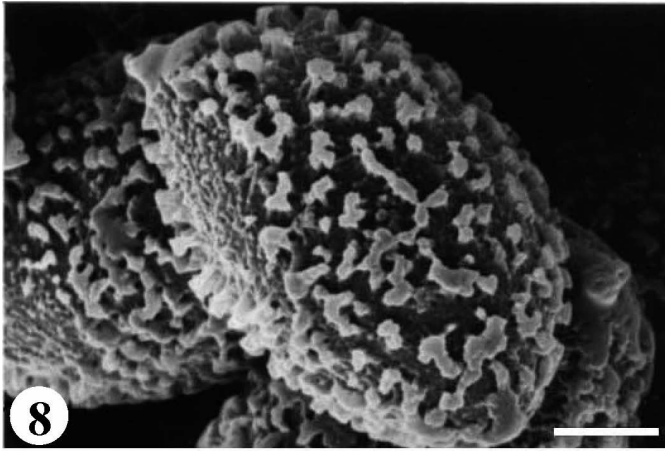
the south-hemspheric taxa forming monophyletic groups. Additionally, the European species *C. acutus* and *C. obtusus* and also *C. conopileus* from tropical India belong to this clade (Peintner et al. 2003). Although our study as well as recent molecular analyses (Høiland and Holst-Jensen 2000; Peintner et al. 2003, 2004) consistently support *Obtusi* as a natural group separated of the *Telamonia* clade discussed below, there are relatively few morphological features that allow a differentiation between *Obtusi* and some members of the *Telamonia* clade. Most *Obtusi* species are characterized by relatively small basidiomes, predominantly ellipsoid to globose hyphal elements in the trama of both pileus and lamellae, ellipsoid spores and hyaline veil hyphae. We expect that once more data on pigment contents of the basidiomes get available, these will be useful markers for a separation of sections *Obtusi* and *Telamonia*.

Telamonia clade (<50/<50)

This group, which was only poorly supported in our metapopulation ML and BIONJ analyses, includes the core group of subgenus *Telamonia* as currently accepted. There is a considerable interspecific variation in the size of the basidiomes. The centre of diversity of this group seems to be the meridional-boreal areas, but according to our molecular analysis the section also includes the south-hemspheric species *C. fulvoconicus* from southern Chile, which is associated with *Nothofagus*. Additional species of this group have been included in "Telamonia II" by Peintner et al. (2001) and "/Telamonia" by Peintner et al. (2003, 2004). All taxa of this group are characterized microscopically by a *pileipellis duplex* with a well-developed hypocutis (especially in species with relatively small basidiomes) and a poorly developed epicutis. At the molecular level, the presence of two characteristic indels in the ITS1 (165 and 21 bp in our alignment) and three indels (13, 15, and 8 bp in our alignment) in the ITS2 regions, respectively, distinguish the species of this clade from the remaining *Cortinarius* species analysed here. Several internal groupings have been introduced for the *Telamonia* clade (Moser 1986; Brandrud et al. 1990–1998; Moënne-Loccoz et al. 1990–2004), and it probably requires studies including a higher number of collections and sequence data from additional genes to shed more light on natural subgroups of this *Cortinarius* complex.

Limitations of our phylogenetic framework

In designing the framework for a more natural classification of the genus *Cortinarius* discussed above, we adopted a conservative position and considered only those groups in our phylogenetic tree that received high branch support or at least can be well characterized on the basis of morphological or microscopical similarities. As can be easily inferred from the phylogenetic tree (Fig. 1), there is a conspicuous number of species included in the present study that following these principles cannot yet be assigned to a clade. Reasons for this situation might be that the respective most closely related species have not been included in this study and (or) the limitation in phylogenetic resolution of the present study. Both aims, increasing the width of species sampling and increasing the phylogenetic resolution of the molecular analyses, the latter by including DNA sequences from other regions of the genome, are central future challenges.

Intraspecific variability of the ITS region

Our molecular analyses showed that the ITS region is highly specific at the species level in *Cortinarius*. In most cases where we found an intraspecific variation of ITS sequences this occurred in taxa that are considered as morphologically variable. We suppose that in most of these cases this indicates that the respective taxa should be assigned to different species rather than to different subspecies or even varieties of one species. More collections of the respective taxa should, however, be sequenced and thoroughly studied with respect to their macromorphology, micromorphology, and ecology before formal taxonomic revisions should be actually made.

Moreover, we found in the tree several species on relatively long branches, for example, *C. callisteus*, *C. austrolimonius* var. *ochrovelatus*, and *C. rubicundulus*. Similar results have been reported by Peintner et al. (2004), using a Bayesian analysis of ITS sequences, for the species *C. callisteus* and *C. tophaceus* and some telamonioid species. The presence of terminal taxa on relatively long branches might either indicate a lack in more closely related species in the sampling used for the present study or locally higher substitution rates in these lineages.

Phylogenetic relationships among major clades

Our phylogenetic analysis (Fig. 1) shows several well-supported lineages in *Cortinarius*. However, we were not able to resolve the relationships among the clades. Nevertheless, there are topological arrangements that, although they appear without significant branch support, may reflect aspects of a natural classification, for example, *Dermocybe*/*Splendidi* uniting species of subgenus *Dermocybe*, and *Obtusi*/*Telamonia* containing telamonioid species appear as closely related in the phylogenetic tree, though without high branch support. The basal position of section *Telamonia* in our phylogenetic tree is conceivable as it correlates with certain morphological/anatomical (*pileipellis duplex* lacking a gelatinous layer), and ontogenetic (pileo-stipitocarpous development of the basidiomes) traits that can be interpreted as plesiomorphic stages.

Distribution of morphological, microscopical and chemical traits among major clades

The currently accepted division of *Cortinarius* into subgenera and groups of lower taxonomic level is predominantly based on macromorphological characters. The clade framework proposed in the present study is in conflict with these subgeneric limitations in many cases. Members of subgenus *Phlegmacium* occur for the most part in the three separate clades *Calochroi*, *Caerulescentes* and *Phlegmacioides*, but also in the smaller clades *Alluti*, *Amarescentes*, *Arguti*, *Percomes*, *Phlegmacium*, *Purpurascentes*, *Scauri*, and *Vulpini*. Part of subgenus *Sericeocybe* was split into the clades *Anomali* and *Telamonia*. Most of the European *Myxacium* species analysed here were placed into the two well-supported separated clades *Delibuti* and *Myxacium*. The northern hemispheric species of subgenus *Dermocybe* formed a well-supported clade (the *Dermocybe* clade as conceived here); part of the southern hemispheric species of this subgenus were placed in the *Splendidi* clade. The *Dermocybe* and *Splendidi* clades appeared as closely related in our phyloge-

netic tree. The European species of subgenus *Telamonia* are distributed over the clades *Obtusi*, *Renidentes*, and *Telamonia*, the latter including most of the species from boreal-meridional areas. The boreal-meridional species of subgenus *Rozites* were placed in the *Rozites* clade, whereas the remaining *Rozites* species analysed here had isolated positions. These results suggest that characters traditionally used for the delimitation of subgenera in *Cortinarius* (Figs. 2–5) are heavily affected with homoplasy. We suggest that the striking morphological resemblance of species from several presumably independent evolutionary lineages reflects in part similar adaptive ecological strategies. The viscosity of the pileus might have been influenced by moist climatic conditions, the evolution of sequestrate basidiomes by dry environments (Thiers 1984). Traits such as stipe shape are possibly correlated with the size of the basidiomes. A marginate stipe base appears in all species of two presumably independent clades (*Calochroi* and *Caerulescentes*) and also in some other species with relatively large basidiomes mainly growing on calcareous soil. The fusiform stipe appears in several species of the clades *Vulpini*, *Pseudotriumphantes* and *Arguti* that mainly grow on soils with high organic content. The anatomy of the pileipellis might be correlated with the size of the basidiomes; a *pileipellis simplex* (Fig. 6) is found in species with larger basidiomes of the clades *Calochroi* (except for *C. aureocalceolatus*), *Cortinarius*, *Heterocliti* and *Phlegmacioides*, whereas the *duplex* type (Fig. 7) is dominant in species with small to medium-sized basidiomes.

Citriform spores are dominant in the phlegmacioid *Calochroi* and *Caerulescentes* clades, subglobose to rounded spores in the *Anomali* clade and ellipsoid spores in the *Dermocybe* clade (Figs. 8–15). With respect to basidiome coloration, we found *Cortinarius* clades with a spectacular interspecific diversity of colours, as discussed above for the *Percomes* clade. On the other hand, we repeatedly observed similar colour patterns among presumably distant *Cortinarius* lineages. We are convinced that the chemistry of basidiome pigments will provide additional taxonomic markers to characterize major *Cortinarius* lineages in the future. However, at the moment pigment data are lacking for most of the relevant taxa to test this hypothesis. Another obviously useful chemical marker to delimit some of the *Cortinarius* lineages (e.g., *Calochroi*, *Dermocybe*, *Phlegmacioides*, *Splendidi* and part of *Percomes*) is the traditionally used bright macro- and microscopical reaction with KOH (Oertel and Laber 1986).

Geographic distribution of *Cortinarius* lineages

Our molecular phylogenetic analysis yields interesting insights in patterns of geographic distribution within some of the proposed *Cortinarius* clades. On the one hand, there are clades such as *Calochroi*, *Dermocybe*, and *Splendidi* that are restricted to the northern or the southern hemisphere, and on the other hand clades that integrate species from a wide latitudinal and longitudinal range (e.g., *Anomali*, *Purpurascentes*, *Rozites*). Hypotheses about the factors that influenced the global distribution patterns and migration of species in *Cortinarius* must take into account the problem of the acquisition of new host trees during geological times. It is of course premature to draw conclusions from these

data, since our analysis is biased toward European species and collections. Sequence data of North American and southern hemispheric species are still scarce, and DNA sequences or thorough morphological/anatomical studies of Asian, Central American and African Cortinarii are lacking. Our results clearly demonstrate that to achieve a better understanding of evolutionary relationships and for the subsequent elaboration of a sound classification system it is imperative to consider the genus *Cortinarius* in a global context.

References

Arnold, N. 1993. Morphologisch-anatomische und chemische Untersuchung der Untergattung *Telamonia* (*Cortinarius*, Agaricales). IHW Verlag, Eching, Germany.

Arnold, N., Besl, H., Bresinsky, A., and Kemmer, H. 1987. Notizen zur Chemotaxonomie der Gattung *Dermocybe* (Agaricales) und zu ihrem Vorkommen in Bayern. Z. Mykol. **53**: 187–194.

Bidaud, A., Moënne-Loccoz, P., and Reumaux, P. 1994. Atlas des Cortinaires, clé génerale des sous-genres, sections, sous-sections et séries. Editions Fédération Mycologique Dauphiné-Savoie, Annecy, France.

Brandrud, T.E., Lindström, H., Marklund, H., Melot, J., and Muskos, S. 1990–1998. *Cortinarius*, flora photographica. Vols. 1 (1990), 2 (1992), 3 (1995), and 4 (1998). German edition. Cortinarius HB, Matfors, Sweden.

Brandrud, T.E. 1996*a*. *Cortinarius* subgenus *Phlegmacium* section *Phlegmacium* in Europe. Descriptive part. Edinb. J. Bot. **53**: 331–400.

Brandrud, T.E. 1996*b*. *Cortinarius* subgenus *Phlegmacium*, section *Phlegmacium* in Europe. A study of character variation and ecology including a numerical analysis of the *C. argutus* complex. Mycol. Res. **100**: 471–485.

Brandrud, T.E. 1998*a*. *Cortinarius* subgenus *Phlegmacium* section *Phlegmacioides* (= *Variicolores*) in Europe. Edinb. J. Bot. **55**: 65–156.

Brandrud, T.E. 1998*b*. *Cortinarius* subgen. *Phlegmacium* sect. *Fulvi* – chemotaxonomy versus morphological taxonomy. Journal des Journées européennes du Cortinaire, **0**: 4–9.

Chambers, S.M., Sawyer, N.A., and Cairney, J.W.G. 1999. Molecular identification of co-occurring *Cortinarius* and *Dermocybe* from southeastern Australian sclerophyll forests. Mycorrhiza, **9**: 85–90.

Clémençon, H. 1971. Amyloide Hyphen und dextrinoide Sporen bei *Rozites caperatus*. Z. Pilzk. **37**: 161–168.

Earle, F.S. 1909. The genera of the North American gill fungi. Bull. N.Y. Bot. Gard. **5**: 373–451.

Fayod, V. 1889. Prodrome d'une histoire naturelle des Agaricinés. Ann. Sci. Nat. Soc. **9**: 181–411.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**: 783–791.

Fries, E.M. 1836–1838. Epicrisis systematis mycologici seu synopsis Hymenomycetum. Uppsala, Sweden.

Fries, E.M. 1878–1884. Icones selectae Hymenomycetum nondum delineatorum. II. Holmiae et Upsaliae.

Gardes, M., and Bruns, D. 1993. ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Mol. Ecol. **2**: 113–118. PMID: 8180733.

Garnica, S., Weiß, M., and Oberwinkler, F. 2003*a*. Morphological and molecular phylogenetic studies in South American *Cortinarius* species. Mycol. Res. **107**: 1143–1156. doi: 10.1017/S0953756203008414. PMID: 14635763.

Garnica, S., Weiß, M., Oertel, B., and Oberwinkler, F. 2003*b*. Phylogenetic relationships of European *Phlegmacium* species (*Cortinarius*, Agaricales). Mycologia, **95**: 1155–1170.

Gascuel, O. 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. Mol. Biol. Evol. **14**: 685–695. PMID: 9254330.

Gasparini, B. 2001. A contribution to the knowledge of *Cortinarius* and allied genera of Southern Tasmania. Australas. J. Mycol. **20**: 49–54.

Gasparini, B. 2006. Contribution to the knowledge of *Cortinarius* and allied genera of Southern Tasmania. Genus *Cortinarius*, subgenus *Phlegmacium*. Australas. J. Mycol. In press.

Gill, M., and Steglich, W. 1987. Pigments of fungi (macromycetes). Progr. Chem. Organic Nat. Prod. **51**: 1–317.

Gruber, I. 1970. Anthrachinonfarbstoffe in der Gattung *Dermocybe* und Versuch ihrer Auswertung für die Systematik. Z. Pilzk. **36**: 95–112.

Hasegawa, M., Kishino, H., and Yano, T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. **22**: 160–174. doi: 10.1007/BF02101694. PMID: 3934395.

Høiland, K. 1984. *Cortinarius* subgenus *Dermocybe*. Opera Bot. **71**: 1–113.

Høiland, K., and Holst-Jensen, A. 2000. *Cortinarius* phylogeny and possible taxonomic implications of ITS rDNA sequences. Mycologia, **92**: 694–710.

Horak, E. 1988. New species of *Dermocybe* (Agaricales) from New Zealand. Sydowia, **40**: 1–80.

Horak, E., and Wood, A.E. 1990. *Cortinarius* Fr. (Agaricales) in Australasia. 1. Subgen. *Myxacium* and subgen. *Paramyxacium*. Sydowia, **42**: 88–168.

Keller, G. 1982. Pigmentationsuntersuchungen bei europäischen Arten aus der Gattung *Dermocybe* (Fr.) Wünsche. Sydowia, **35**: 110–126.

Keller, G., and Ammirati, J. 1983. Chemotaxonomic significance of anthraquinone derivatives in North American species of *Dermocybe*, section *Sanguineae*. Mycotaxon, **18**: 357–377.

Keller, G., and Ammirati, J. 1995. Chemotaxonomic studies on the pigmentation of North American species of *Dermocybe* (Fr.) Wünsche, section *Dermocybe* and related species. Beih. Sydowia, **10**: 127–136.

Keller, G., Moser, M., Horak, E., and Steglich, W. 1988. Chemotaxonomic investigations of species of *Dermocybe* Fr. Wünsche (Agaricales) from New Zealand, Papua New Guinea and Argentina. Sydowia, **40**: 168–187.

Lemmon, A.R., and Milinkovitch, M. 2002. The metapopulation genetic algorithm: an efficient solution for the problem of large phylogeny estimation. Proc. Natl. Acad. Sci. U.S.A., **99**: 10516–10521. doi: 10.1073/pnas.162224399. PMID: 12142465.

Liu, Y.L., Rogers, S.O., and Ammirati, J.F. 1997. Phylogenetic relationships in *Dermocybe* and related *Cortinarius* taxa based on nuclear ribosomal DNA internal transcribed spacers. Can. J. Bot. **75**: 519–532.

Meixner, A. 1975. Chemische Farbreaktionen von Pilzen. J. Cramer, Vaduz, Germany

Moënne-Loccoz, P., Reumaux, P., and Bidaud, A. 1990–2004. Atlas des Cortinaires 1–14. Editions Fédération Mycologique Dauphiné-Savoie. Annecy, France.

Moser, M. 1960. Die Gattung *Phlegmacium* (Schleimköpfe). Die Pilze Mitteleuropas, Bd. IV. J. Klinkhardt, Bad Heilbrunn, Germany.

Moser, M. 1969–1970. *Cortinarius* Fr., Untergattung *Leprocybe* subgen. nov., die Rauhköpfe. Vorstudien zu einer Monographie. Z. Pilzk. **35**(3–4): 213–248; **36**(1–2): 19–39.

Moser, M. 1983. Die Röhrlinge und Blätterpilze. *In* Kleine Kryptogamenflora, Band II b/2. 5th ed. *Edited by* H. Gams. G. Fischer, Stuttgart.

Moser, M. 1986. *Cortinarius* and *Dermocybe*. *In* The Agaricales in modern taxonomy. 4th ed. *Edited by* R. Singer. Koeltz Scientific Books, Koenigstein, Germany. pp. 618–656.

Moser, M., and Ammirati, J.F. 1996. Studies in North American Cortinarii II. Interesting and new species collected in the North Cascade Mountains, Washington. Mycotaxon, **58**: 387–412.

Moser, M., and Ammirati, J.F. 1997. Studies on North American Cortinarii IV. New and interesting *Cortinarius* species (subgenus *Phlegmacium*) from oak forests in Northern California. Sydowia, **49**: 25–48.

Moser, M., and Ammirati, J.F. 1999. Studies in North American Cortinarii V. New and interesting Phlegmacia from Wyoming and the Pacific Northwest. Mycotaxon, **72**: 289–321.

Moser, M., and Horak, E. 1975. *Cortinarius* Fr. und nahe verwandte Gattungen in Südamerika. Beih. Nov. Hed. **52**: 1–628.

Moser, M., and Peintner, U. 2002*a*. Die phylogenetischen Beziehungen der *Cortinarius aureopulverulentus* Gruppe. J.J.E.C. **4**: 28–38.

Moser, M., and Peintner, U. 2002*b*. The species complex *Cortinarius scaurus – C. herpeticus* based on morphological and molecular data. Micol. Vegetazione Mediterr. **17**: 3–17.

Moser, M., McKnight, K.H., and Sigl, M. 1994. The genus *Cortinarius* (Agaricales) in the Greater Yellowstone area. Mycorrhizal host associations and taxonomic considerations. *In* Plants and their environments. Proceedings of the First Biennial Scientific Conference on the Greater Yellowstone Ecosystem. *Edited by* D.G. Despain. US Department of the Interior National Park Service, Natural Resources Publications Office, Denver, Colo. pp. 239–246.

Moser, M., McKnight, K.H., and Ammirati, J.F. 1995. Studies on North American Cortinarii I. New and interesting taxa from the Greater Yellowstone area. Mycotaxon, **55**: 301–346.

Nussbaum, F., Spiteller, P., Rüth, M., Steglich, W., Wanner, G., Gamblin, B., Stievano, L., and Wagner, F.E. 1998. An iron(III)-catechol complex as a mushroom pigment. Angew. Chem. Int. Ed. Engl. **37**: 3292–3295.

O'Donnell, K. 1993. *Fusarium* and its near relatives. *In* The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. *Edited by* D.R. Reynolds and J.W. Taylor. CAB International, Wallingford, UK. pp. 225–233.

Oertel, B. 1984. Untersuchungen zur Konstitution von Dihydroanthrachinonen und Angaben zu ihrer Verbreitung in Pilzen. Ph.D. dissertation. Mathematisch-Naturwissenschaftliche Fakultät, Rheinische Friedrich-Wilhelms-Universität, Bonn.

Oertel, B., and Laber, D. 1986. Die Laugenreaktion an der Unterseite der Stielknolle bei Fruchtkörpern der Gattung *Cortinarius*,

Untergattung *Phlegmacium* (Agaricales). Z. Mykol. **52**: 139–154.

Peintner, U., Bougher, N.L., Castellano, M.A., Moncalvo, J.M., Moser, M.M., Trappe, J.M., and Vilgalys, R. 2001. Multiple origins of sequestrate fungi related to *Cortinarius* (Cortinariaceae). Am. J. Bot. **88**: 2168–2719.

Peintner, U., Horak, E., Moser, M., and Vilgalys, R. 2002. Phylogeny of *Rozites, Cuphocybe* and *Rapacea* inferred from ITS and LSU rDNA sequences. Mycologia, **94**: 620–629.

Peintner, U., Moser, M., Thomas, K.A., and Manimohan, P. 2003. First records of ectomycorrhizal *Cortinarius* species (Agaricales, Basidiomycetes) from tropical India and their phylogenetic position based on rDNA ITS sequences. Mycol. Res. **107**: 485–494. doi: 10.1017/S0953756203007585. PMID: 12825522.

Peintner, U., Moncalvo, J.-M., and Vilgalys, R. 2004. Toward a better understanding of the infrageneric relationships in *Cortinarius* (Agaricales, Basidiomycota). Mycologia, **96**: 1042–1058.

Rambaut, A. 1996. Se-Al. Sequence alignment editor. Department of Zoology, University of Oxford, Oxford, UK.

Ricken, A. 1915. Die Blätterpilze (Agaricaceae) Deutschlands und der angrenzenden Länder, besonders Oesterreichs und der Schweiz. Vols. 1 and 2. *Edited by* Theodor Oswald Weigel. Leipzig, Germany.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425. PMID: 3447015.

Seidl, M.T. 2000. Phylogenetic relationships within *Cortinarius* subgenus *Myxacium*, sections *Defibulati* and *Myacium.* Mycologia, **92**: 1091–1102.

Soop, K. 1999. Cortinarioid fungi of New Zealand. Karl Soop, Éditions Scientrix, Stockholm, Sweden.

Soop, K. 2001. Contribution á l'étude de la mycoflore cortinarioïde de la Nouvelle-Zélande. Bull. Soc. Mycol. Fr. **117**: 91–132.

Steglich, W., and Oertel, B. 1985. Untersuchungen zur Konstitution und Verbreitung der Farbstoffe von *Cortinarius*, Untergattung *Phlegmacium* (*Agaricales*). Sydowia, **37**: 284–295.

Steglich, W., Kopanski, L., and Wolf, M. 1984. Indolalkaloide aus dem Blätterpilz *Cortinarius infractus* (Agaricales). Tetrahedron Lett. **25**: 2341–2344. doi: 10.1016/S0040-4039(01)80250-1.

Swofford, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Mass.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **24**: 4876–4882.

Thiers, H.D. 1984. The secotioid symdrome. Mycologia, **76**: 1–8.

Weiß, M., Yang, Z.L., and Oberwinkler, F. 1998. Molecular phylogenetic studies in the genus *Amanita.* Can. J. Bot. **76**: 1170–1179.