

## Phylogenetic relationships of European *Phlegmacium* species (*Cortinarius*, Agaricales)\*

S. Garnica<sup>1</sup>

M. Weiß

*Lehrstuhl für Spezielle Botanik und Mykologie,  
Botanisches Institut, Universität Tübingen, Auf der  
Morgenstelle 1, D-72076 Tübingen, Germany*

B. Oertel

*Institut für Gartenbauwissenschaft, Universität Bonn,  
Auf dem Hügel 6, D-53121 Bonn, Germany*

F. Oberwinkler

*Lehrstuhl für Spezielle Botanik und Mykologie,  
Botanisches Institut, Universität Tübingen, Auf der  
Morgenstelle 1, D-72076 Tübingen, Germany*

**Abstract:** Phylogenetic relationships of 54 European *Phlegmacium* species, including members of most of the sections of classical systematics, were studied, integrating macro-, micromorphological and chemical characters of the basidiomes, as well as molecular phylogenetic analysis of nuclear rDNA sequences. Microscopical structures of the basidiomes were studied by light microscopy. Basidiospore morphology was examined by scanning electron microscopy. Internal-transcribed spacers (ITS 1 and 2, including the 5.8S) and the D1/D2 (LSU) regions of nuclear rDNA were sequenced and analyzed with a Bayesian Markov chain Monte Carlo approach. Many subgroups detected by the molecular analysis are related to groups known from classical systematical concepts. Among others, these subgroups were significantly supported: i) a group containing most of the members of section *Fulvi* ss. Brandrud and the species *Cortinarius arcuatorum*, *C. dibaphus* and *C. multiformis*; ii) a group comprising taxa of section *Calochroi* ss. Brandrud and the species *C. fulvocitrinus* and *C. osmophorus*; iii) a group containing species of section *Glaucopodes* ss. Brandrud and *C. caerulescens*; iv) a group including members of section *Phlegmacioides* ss. Brandrud; v) a group that includes the species *C. cephalixus*, *C. nancaiensis* and *C. mussivus*. Stipe shape, color of flesh, pigment contents, KOH reaction on pileipellis and gelatinous layer, degree of development of a gelatinous layer on the pileipellis, and pileipellis structure

were useful characters in delimiting subgroups in *Phlegmacium*, while basidiospore morphology was significant at species level. With the exception of *C. glaucopus*, *C. infractus* and *C. scaurus*, ITS and D1/D2 sequences obtained from collections of the same species from different geographical origins showed very little variation. Our molecular and morphological analyses suggest revisions of the traditional concepts of the subgenus *Phlegmacium* in Europe.

**Key words:** D1/D2 domains, Europe, ITS, LSU, molecular phylogeny, morphology, nuc rDNA, systematics

### INTRODUCTION

*Phlegmacium* Fr., a subgenus of *Cortinarius*, includes species with relatively fleshy basidiomes with vivid colors, a viscid to glutinous pileus surface and a dry stipe. Many *Phlegmacium* species have a wide distribution in Europe and occur in ectomycorrhizal association with coniferous and deciduous trees. Some species are supposed to be even more widespread and reach North America (Moser et al 1994, 1995; Moser and Ammirati 1996, 1997, 1999). On the other hand, other *Phlegmacium* species show a limited distribution, mainly due to high host-tree specificity, or specific climatic or edaphic requirements.

Since Fries (1821) introduced the name *Phlegmacium* as a tribus of the genus *Agaricus*, many mycologists have contributed to the systematics and taxonomy of the *Phlegmacium* species in Europe, grouping them in a separate genus (e.g., Wünsche 1877, Fayod 1889, Earle 1909, Ricken 1915, Moser 1960) or in a subgenus of *Cortinarius* (e.g., Fries 1836–1838, 1878–1884; Moser 1983; Moënne-Loccoz et al 1990–2001; Bidaud et al 1994; Brandrud et al 1990–1998). Modern classification systems recognize *Phlegmacium* as a subgenus of *Cortinarius*. Species delimitations in *Phlegmacium* traditionally have been based almost exclusively on “field recognition”, e.g., coloration of pileus and lamellae, as well as macrochemical tests combined with basidiospore morphology (e.g., Moser 1960, 1983, 1986; Moënne-Loccoz et al 1990–2001; Bidaud et al 1994). Attempts to increase the number of characters used for taxonomic purposes in *Phlegmacium* have been made by Oertel (1984), Oertel and Laber (1986), Steglich et al

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<sup>1</sup> Corresponding author. E-mail: sigisfredo.garnica@uni-tuebingen.de

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(1984), Steglich and Oertel (1985), Gill and Steglich (1987) and Brandrud (1998b); all of these authors included pigment chemistry of the basidiomes. Brandrud (1996a, b, 1998a) reported the taxonomic significance of microscopical characters referring to veil and pileipellis structure for the delimitation of some sections in *Phlegmacium*.

Various classification systems have been proposed for *Phlegmacium* in Europe. Moser (1960), who monographed most of the *Phlegmacium* species in this region, recognized these sections: *Amarescentes*, *Caerulescentes*, *Calochroi*, *Fulvi*, *Laeticolores*, *Phlegmacium* and *Triumphantes*. Moser (1983) defined a new section *Tenuis* and changed the name of section *Laeticolores* to *Scauri*. Bidaud et al (1994) and Moënnelocoz et al (1990–2001) included some taxa from the subgenera *Myxadium* and *Sericeocybe* in *Phlegmacium* and recognized the sections *Caerulescentes*, *Claricolores*, *Delibuti*, *Fulgentes*, *Glauropodes*, *Laeticolores*, *Multiformes*, *Paties*, *Phlegmacium* and *Thalliophili*. Brandrud et al (1990–1998) and Brandrud (1996a, b, 1998a, b) made several emendations of previous classification systems. They incorporated a large quantity of microscopic and chemical characters and used numerical analyses to split *Phlegmacium* into sections *Caerulescentes*, *Calochroi*, *Elastici*, *Fulvi*, *Glauropodes*, *Infracti*, *Multiformes*, *Phlegmacioides*, *Phlegmacium*, *Scauri* and *Subtorti*. Molecular analyses based on the ITS (1 and 2) and 5.8S regions of nuclear rDNA recently have been applied to a limited number of European *Phlegmacium* species (Høiland and Holst-Jensen 2000, Peintner et al 2001).

Using original morphological and molecular data obtained from Friesian's and Henry's *Phlegmacium* species, we have tried to evaluate macro- and micro-morphological as well as chemical characters, by comparing them through molecular phylogenetic analyses. Our species sampling includes members of most of the *Phlegmacium* sections recognized in traditional systematics. To get information on the degree of intraspecific variation of the molecular data, we mostly sequenced several collections of the same *Phlegmacium* species from different sites in Europe. Our phylogenetic hypotheses are discussed with current classification systems applied to *Phlegmacium* species in Europe.

#### MATERIAL AND METHODS

*Material studied.*—A total of 86 collections, representing 54 European *Phlegmacium* species, were included in this study. Collection sites, host trees, locations of vouchers and GenBank accession numbers of the *Phlegmacium* species used in the morphological and molecular analyses are listed in TABLE I. We chose *Laccaria amethystina* Cooke, GenBank

accession No. AF539737 (Garnica et al 2003) as outgroup species for the present molecular phylogenetic analysis, according to results obtained with D1/D2 sequences from a broad sampling of Agaricales (S. Garnica and M. Weiß unpubl) and multigene analyses by Binder and Hibbett (2002). Detailed macroscopical descriptions were made from fresh basidiomes and complemented with color photographs. Macrochemical tests with 40% KOH solution were performed on pileus surface, context and stipe base of the basidiomes. Identification of the collections were based on taxonomic keys of Moser (1960, 1983) and Brandrud (1996a, b, 1998a).

TABLE II summarizes three current classification systems for European *Phlegmacium* species, restricted to the taxa that are examined in this study.

*Microscopical analyses.*—Microscopical structures were studied from dried material mounted in 3% KOH. Freehand sections from the pileus and hymenophoral trama were made under a dissecting microscope with a razorblade. Drawings (FIGS. 9–10) were made from longitudinal sections of the pileus stained with 0.1% Congo red solution with the aid of a camera lucida with a 40× objective. The surface of the basidiospores was studied by scanning electron microscopy (SEM), according to the methods of Garnica et al (2002) (FIGS. 11–18).

*Molecular analyses.*—Genomic DNA was isolated from lamellar fragments (approx. 100 mg), according to the method described by Weiß and Oberwinkler (2001). The ITS region (including the gene coding for the 5.8S ribosomal subunit) and the D1/D2 region of the ribosomal large subunit (LSU) were amplified with polymerase chain reactions (PCR, Mullis and Faloona 1987) with the primer combinations ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3', Gardes and Bruns 1993)/NL4 (5'-GGTCCGTGTTTCAAGACGG-3', O'Donnell 1993); in some cases, we alternatively used the primer combinations ITS1F/ITS4 (5'-TCCTCCGCTTATTGATATGC-3', White et al 1990) and 5.8SR (5'-TCGATGAAGAACGCAGCG-3')/LR3 (5'-CCGTGTTTCAAGACGGG-3', Vilgalys and Hester 1990). PCR concentrations of the reaction components and cycling parameters were as indicated in Weiß et al (1998). Amplified PCR products were checked on an agarose gel (0.7%), stained with ethidium bromide and visualized under UV light. PCR products were purified with the QIAquick™ kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. Cycle sequencing was performed with the ABI PRISM™ BigDye™ cycle sequencing kit (Applied Biosystems/Perkin Elmer) and the primers ITS1F, ITS4, NL4 and NLMW1 (5'-TCAATAGCGGAGGAAAAGA-3', Sampaio et al 2002), sometimes complemented with the primers NL2Cor (5'-CTCTTTCCAAAGTTCTTTTCA-3'), a modification of a primer given by Boekhout et al [1995]), 5.8SR, and LR3. The sequences were produced with an automated sequencer ABI 373A (Applied Biosystems/Perkin Elmer).

An alignment of 86 sequences, representing 54 *Phlegmacium* species and *Laccaria amethystina* as outgroup species, was made with the MegAlign module of the Lasergene software system (DNASTAR Inc.), followed by manual ad-

justments in Se-Al (Rambaut 1996). Sequence alignments may be obtained from TreeBase (<http://treebase.bio.buffalo.edu/treebase/>). Regions with ambiguous alignments, which occupied positions 1–60, 196–209, 236–249, 351–358, 537–567, 581–590, 599–613, 656–666, 705–724, 760–777 and 1412–1422 in our data matrix, were excluded for the phylogenetic analysis.

To estimate the phylogenetic relationships of the *Phlegmacium* species, the DNA alignment was analyzed using a Bayesian approach based on Markov chain Monte Carlo (MCMC; Larget and Simon 1999), as implemented in the computer program MrBayes 2.01 (Huelsenbeck and Ronquist 2001). In contrast to the maximum-likelihood method (Felsenstein 1981), in which the probability of the DNA alignment conditional on phylogenetic trees (the “likelihood” of the phylogenetic trees) is maximized, the Bayesian MCMC approach allows estimation of the a posteriori probability of phylogenetic trees, i.e., the probability that a tree is the true phylogenetic tree given the DNA alignment. Because the posterior probability distribution of the tree space is analytically inaccessible, the method uses a Monte Carlo technique to collect a large representative sample of phylogenetic trees from the tree space, from which the posterior probabilities can be estimated. By summing posterior probabilities of those trees in which a group of taxa is monophyletic it also is possible to estimate the a posteriori probability for the monophyly of given groups, i.e., the probability that a group is monophyletic given the DNA alignment. The power of this method to reconstruct phylogenetic relationships efficiently has been demonstrated by Murphy et al (2001) for mammalian phylogeny and by Maier et al (2003) and Garnica et al (2003) for several fungal groups. To improve mixing of the chain, we ran four incrementally heated simultaneous Monte Carlo Markov chains (Metropolis-coupling technique; see Huelsenbeck et al 2002) over 2 000 000 generations, using the general time-reversible model of DNA substitution with gamma-distributed substitution rates (see Swofford et al 1996), random starting trees and default starting parameters of the DNA substitution model. Trees were sampled every 100 generations, resulting in an overall sampling of 20 000 trees. From those trees that were sampled after the process had reached stationarity, a 50% majority-rule consensus tree was computed to get estimates for a posteriori probabilities. Branch lengths of this consensus tree were estimated with PAUP 4.0b10 (Swofford 2001) using maximum likelihood. The Bayesian MCMC phylogenetic analysis was repeated three times, always using random starting trees, on a Macintosh G4 computer to test the independency of the results from topological priors (Huelsenbeck et al 2002).

## RESULTS

*Macroscopical characters.—Habit.* FIGURES 1–8 show some selected basidiome habits of *Phlegmacium* species studied. Size of the basidiomes varied from medium to relatively large and robust. Basidiomes of *Phlegmacium* species collected on oligotrophic to mesotrophic soils were medium size (e.g., *C. scaurus*

and *C. subtortus*), whereas the species from eutrophic-calciphilous soils were characterized by fleshy and relatively robust basidiomes.

*Macroscopical characters.—Pileus surface.* A large intra- and interspecific variation concerning the degree of viscosity and the texture of the pileus surface was found in *Phlegmacium* spp. Both features can be influenced by the age of basidiomes and environmental factors. The degree of viscosity of the pileus surface varied from slightly glutinous (viscid) to dry (*C. balteatocumatilis*, *C. caerulea*, *C. cumatilis*, *C. coalescens*, *C. variicolor*) and glutinous in the remaining species analyzed. The texture of the pileus surface ranged from glabrous in a large number of species, fibrillose in *C. anserinus*, *C. dionysae*, *C. glaucopus*, *C. infractus*, *C. lustratus*, *C. multiformis*, *C. coalescens*, *C. variicolor* and *C. vulpinus* to squamose in various *Phlegmacium* spp. Very small scales in the center of the pileus characterized *C. calochrous*, *C. calochrous* var. *coniferarum*, *C. cephalixus*, *C. citrinililacinus*, *C. prasinus*, *C. citrinus*, *C. elegantior*, *C. fulvocitrinus*, *C. nanceiensis*, *C. splendens* and *C. meinhardii*. Abundant ochraceous-brown veil remnants, forming more or less concentric scales toward the pileus margin, characterized *C. triumphans* and *C. saginus*. A white fibrillose zone toward the pileus margin characterized *C. vulpinus*. In mature basidiomes of *C. praestans* the pileus margin becomes wrinkled.

*Macroscopical characters.—Stipe.* The shape of the stipe base varied from attenuate to subradicating (*C. turmalis*, *C. vulpinus*), cylindrical to clavate (*C. cephalixus*, *C. cumatilis*, *C. infractus*, *C. lustratus*, *C. coalescens*, *C. mussivus*, *C. porphyropus*, *C. saginus*, *C. scaurus*, *C. triumphans*, *C. variiformis*, and *C. varius*) and bulbous (rounded, submarginate to marginate) in the remaining taxa studied (see FIG. 19). Scaly to floccose ring-like zones that become ocher-brown in age were found in the lower part of the stipe in *C. triumphans*, *C. saginus* and *C. vulpinus*. Ocher ring-like velum zones were observed in *C. anserinus*, *C. varius* and *C. variiformis*; these were brown in *C. nanceiensis* and slightly blue in *C. praestans*.

*Macroscopical characters.—Coloration.* The basidiomes of *Phlegmacium* species showed a wide spectrum of bright colors. A large degree of inter- and infraspecific variation of basidiome coloration was observed, depending on the age of the basidiome and environmental factors (e.g., exposure to light). We have summarized the coloration of the lamellae (in young and near mature basidiomes) and flesh (context) of fresh material in these three main categories: i) white to ocher, ii) yellow to greenish (ol-

TABLE I. European *Phlegmacium* species included for morphological and molecular analyses

Species	Host tree and collection site	Herbarium	GenBank No.
<i>Cortinarius anserinus</i> (Velen.) R. Hry.	<i>Fagus sylvatica</i> , Gerolstein-Gees, Germany	TUB 011404	AY174806
	<i>Fagus sylvatica</i> , Eßlingen, Germany	TUB 011436	AY174807
	<i>Fagus sylvatica</i> , Tondorf, Germany	TUB 011459	AY174805
<i>C. arcuatorum</i> R. Hry.	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011403	AY174824
	<i>Fagus sylvatica</i> and <i>Pinus</i> sp., near Tübingen, Germany	TUB 011421	AY174822
	<i>Fagus sylvatica</i> , Buir, Germany	TUB 011447	AY174823
<i>C. atrovirens</i> Kalchbr.	<i>Picea abies</i> and <i>Abies alba</i> , Locherdorf, Germany	UL 96/81	AY174848
<i>C. balteatocumatilis</i> (R. Hry) ex P.D. Orton	<i>Fagus sylvatica</i> , Piroulette, France	TUB 011440	AY174801
<i>C. boudieri</i> R. Hry. ex R. Hry.	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011402	AY174860
	<i>Fagus sylvatica</i> and <i>P. abies</i> , Nohn, Germany	TUB 011424	AY174861
<i>C. caerulescens</i> (Schaeff.) Fr.	<i>Fagus sylvatica</i> , Wöllmisse, Germany	UL 98/88	AY174863
	<i>Fagus sylvatica</i> , Loogh, Germany	TUB 011423	AY174862
<i>C. caesiocortinatus</i> Schaeff.	<i>Fagus sylvatica</i> , Gerolstein-Gees, Germany	TUB 011400	AY174809
<i>C. calochrous</i> Fr.	<i>Fagus sylvatica</i> , Gerolstein-Gees, Germany	TUB 011398	AY174838
<i>C. calochrous</i> var. <i>coniferarum</i> (M.M. Moser) Quadr.	<i>Picea abies</i> , Oberjoch, Germany	TUB 011385	AY174842
<i>C. cephalixus</i> Fr.	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011395	AY174783
	<i>Fagus sylvatica</i> , Eßlingen, Germany	TUB 011444	AY174784
	<i>Fagus sylvatica</i> and <i>Pinus</i> sp., near Tübingen, Germany	TUB 011391	AY174786
<i>C. cereifolius</i> (M.M. Moser) M.M. Moser	<i>Fagus sylvatica</i> , Buir, Germany	TUB 011426	AY174847
<i>C. citrinolilacinus</i> (M.M. Moser) M.M. Moser	<i>Fagus sylvatica</i> , Urft, Germany	TUB 011442	AY174830
<i>C. citrinus</i> J.E. Lange ex P.D. Orton	<i>Fagus sylvatica</i> , Wöllmisse, Germany	UL 99/87	AY174820
	<i>Fagus sylvatica</i> , Gerolstein-Gees, Germany	TUB 011407	AY174821
	<i>Fagus sylvatica</i> , Loogh, Germany	TUB 011452	AY174825
<i>C. claroflavus</i> R. Hry.	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011427	AY174852
<i>C. coalescens</i> Kärcher & Seibt.	<i>Fagus sylvatica</i> , Weyer, Germany	TUB 011455	AY174794
<i>C. cumatilis</i> Fr.	<i>Picea abies</i> , Oberjoch, Germany	TUB 011417	AY174812
<i>C. cupreorufus</i> Brandr.	<i>Picea abies</i> , Tannheim, Austria	TUB 011418	AY174831
<i>C. dibaphus</i> Fr.	<i>Picea abies</i> , Levier, France	TUB 011437	AY174819
<i>C. dionysae</i> R. Hry.	<i>Fagus sylvatica</i> , Weyer, Germany	TUB 011450	AY174813
<i>C. elegantior</i> (Fr.) Fr.	<i>Picea abies</i> , Oberjoch, Germany	TUB 011394	AY174851
	<i>Picea abies</i> , Oberjoch, Germany	TUB 011388	AY174850
<i>C. flavovirens</i> R. Hry.	<i>Fagus sylvatica</i> , Buir, Germany	TUB 011454	AY174841
<i>C. fulvocitrinus</i> Schaeff. ex Brandr.	<i>Fagus sylvatica</i> , Oos, Germany	TUB 011434	AY174828
<i>C. glaucopus</i> (Schaeff.: Fr.) Fr.	<i>Picea abies</i> , Oberjoch, Germany	TUB 011414	AY174787
	<i>Fagus sylvatica</i> , Nohn, Germany	TUB 011397	AY174785
<i>C. infractus</i> (Pers.: Fr.) Fr.	<i>Picea abies</i> , Oberjoch, Germany	TUB 011384	AY174779
	<i>Fagus sylvatica</i> , Gerolstein-Gees, Germany	TUB 011396	AY174780
	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011441	AY174781
<i>C. ionochlorus</i> Maire	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011430	AY174834
<i>C. lustratus</i> Fr.	<i>Fagus sylvatica</i> , Bücheneck, Germany	UL 98/92	AY174853
<i>C. meinhardii</i> Bon	<i>Picea abies</i> , Birresborn, Germany	TUB 011443	AY174840
	<i>Picea abies</i> , Oberjoch, Germany	TUB 011390	AY174839
<i>C. multiformis</i> Fr. ss. M.M. Moser	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011410	AY174846
	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011458	AY174844
<i>C. mussivus</i> (Fr.) Melot	<i>Picea abies</i> , Oberjoch, Germany	TUB 011412	AY174814
<i>C. nanceiensis</i> Maire	<i>Fagus sylvatica</i> and <i>Picea abies</i> , Oberjoch, Germany	TUB 011389	AY174855
	<i>Picea abies</i> , Taubenberg, Rinnen, Germany	TUB 011422	AY174856

TABLE I. Continued

Species	Host tree and collection site	Herbarium	GenBank No.
<i>C. odoratus</i> (Joguet ex M.M. Moser) M.M. Moser	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011438	AY174836
<i>C. odorifer</i> Britzelm.	<i>Picea abies</i> , Oberjoch, Germany	TUB 011383	AY174817
<i>C. osmophorus</i> P.D. Orton	<i>Fagus sylvatica</i> , Loogh, Germany	TUB 011399	AY174815
	<i>Fagus sylvatica</i> , Loogh, Germany	TUB 011445	AY174816
<i>C. porphyropus</i> (Alb. & Schw.) Fr.	Forest with <i>Quercus</i> , <i>Salix</i> , <i>Populus</i> , Daun, Germany	TUB 011451	AY174854
<i>C. praestans</i> (Cordier) Gillet	<i>Fagus sylvatica</i> , Weyer, Germany	TUB 011460	AY174804
	<i>Fagus sylvatica</i> , Dußlingen, Germany	TUB 011420	AY174803
	<i>Fagus sylvatica</i> , near Tübingen, Germany	TUB 011448	AY174802
<i>C. prasinus</i> Fr. ss. Konr. & Maubl.	<i>Fagus sylvatica</i> and <i>Picea abies</i> , Nohn, Germany	TUB 011431	AY174835
	<i>Fagus sylvatica</i> and <i>Picea abies</i> , Nohn, Germany	TUB 011446	AY174843
<i>C. provençalis</i> M.M. Moser Maire	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011439	AY174818
<i>C. purpurascens</i> Fr.	<i>Picea abies</i> , Gerolstein-Gees, Germany	TUB 011401	AY174858
<i>C. pseudofulmineus</i> R. Hry. ex R. Hry.	<i>Fagus sylvatica</i> and <i>Carpinus</i> , Coloman, Germany	TUB 011433	AY174837
<i>C. pseudonapus</i> R. Hry.	<i>Picea abies</i> , Birresborn, Germany	TUB 011429	AY174864
<i>C. rufoolivaceus</i> Fr.	<i>Fagus sylvatica</i> and <i>Quercus</i> sp., Nohn, Germany	TUB 011405	AY174845
	<i>Fagus sylvatica</i> , Buir, Germany	TUB 011463	AY174849
<i>C. saginus</i> (Fr.: Fr.) Fr.	<i>Pinus</i> sp., Tübingen, Germany	TUB 011419	AY174797
	<i>Picea abies</i> , Sistig, Germany	TUB 011425	AY174800
<i>C. scaurus</i> Fr.	Mixed forest, Birresborn, Germany	TUB 011456	AY174808
	<i>Picea abies</i> , Oberstadt, Germany	TUB 011387	AY174810
<i>C. sodagnitus</i> R. Hry.	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011428	AY174829
<i>C. splendens</i> R. Hry.	<i>Fagus sylvatica</i> , Gerolstein-Gees, Germany	TUB 011411	AY174833
	<i>Fagus sylvatica</i> , Eßlingen, Germany	TUB 011432	AY174832
<i>C. subtortus</i> (Pers. ex Fr.) Fr.	<i>Picea abies</i> , Freudenstadt, Germany	TUB 011382	AY174857
	<i>Picea abies</i> , Imst, Austria	TUB 011386	AY174859
<i>C. triumphans</i> (Fr.) Fr.	<i>Betula</i> sp., Ramersbach, Germany	TUB 011461	AY174799
	<i>Betula</i> sp., Sonnenberg, Thüringen, Germany	UL 96/98	AY174798
<i>C. turmalis</i> Fr.	<i>Picea abies</i> , Oberjoch, Germany	TUB 011393	AY174782
<i>C. varicolor</i> (Pres.: Fr.) Fr.	<i>Picea abies</i> , Oberjoch, Germany	TUB 011416	AY174795
	<i>Picea abies</i> , Oberjoch, Germany	TUB 011415	AY174793
	<i>Picea abies</i> , Üxheim, Germany	TUB 011462	AY174796
<i>C. variiformis</i> Malençon	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011409	AY174791
<i>C. varius</i> Fr.	<i>Picea abies</i> , Oberjoch, Germany	TUB 011392	AY174792
	<i>Picea abies</i> , Imst, Austria	TUB 011413	AY174790
<i>C. viridocaeruleus</i> Chev. & R. Hry.	<i>Fagus sylvatica</i> , Eschweiler, Germany	TUB 011408	AY174788
	<i>Quercus</i> sp., Münster-Nienberg, Germany	TUB 011435	AY174789
<i>C. vulpinus</i> (Velen.) R. Hry.	<i>Fagus sylvatica</i> , Gerolstein-Gees, Germany	TUB 011406	AY174811
<i>C. xanthophyllus</i> Cooke	<i>Fagus sylvatica</i> , Weyer, Germany	TUB 011457	AY174827
	<i>Fagus sylvatica</i> , Weyer, Germany	TUB 011453	AY174826

TUB, Herbarium Tubingense, University of Tübingen, Germany; UL, private Herbarium U. Luhmann.

ive) and iii) violet to bluish (purple) (for flesh coloration see FIG. 19).

**Macroscopical characters.**—*Odor.* The *Phlegmacium* species *C. lustratus*, *C. dionysae* and *C. flavovirens* are characterized by a distinctive farinaceous odor, *C. osmophorus* and *C. odoratus* by a sweet odor such as *Citrus* blossoms, *C. odorifer* by aniseed odor and *C. mussivus* by an apple-like odor later becoming unpleasant; an unpleasant odor reminiscent of rotten potatoes characterizes *C. claroflavus* and a soil-like odor *C. varicolor*.

**Macroscopical characters.**—*Macrochemical reaction.* Many *Phlegmacium* species showed a distinctive color reaction with KOH on pileus surface, context and stipe base. The species *C. boudieri*, *C. scaurus* and *C. flavovirens* did not show any color reaction with KOH. The context and lamellae of *C. scaurus*, *C. porphyropus* and *C. purpurascens* turned purple when touched or scratched.

**Microscopical characters.**—*Gelatinous layer.* In all *Phlegmacia* studied, the outer layer of the pileus was

TABLE II. Current classification systems of European *Phlegmacium* species, restricted to the investigated species.

Moser (1960, 1983, 1986)	Brandrud et al (1990–1998), Brandrud 1996a, b, 1998a, b.	Moëgne-Locoz et al (1990–2001)
Section <i>Amarescentes</i>	Section <i>Caerulescentes</i>	Section <i>Caerulescentes</i>
<i>C. infractus</i>	<i>C. caerulescens</i>	Subsection <i>Caerulescentes</i>
<i>C. subtortus</i>	Section <i>Calochroi</i>	<i>C. boudieri</i>
Section <i>Calochroi</i>	<i>C. calochrous</i>	<i>C. caerulescens</i>
Subsection <i>Calochroi</i>	<i>C. calochrous</i> var. <i>coniferarum</i>	Subsection <i>Praestantes</i>
<i>C. arcuatorum</i>	<i>C. dibaphus</i>	<i>C. cumatilis</i>
<i>C. caesiocortinatus</i>	<i>C. sodagnitus</i>	<i>C. praestans</i>
<i>C. calochrous</i>	Section <i>Fulvi</i>	Subsection <i>Sodagniti</i>
<i>C. calochrous</i> var. <i>coniferarum</i>	Subsection <i>Atrovirentes</i>	<i>C. arcuatorum</i>
<i>C. citrinolilacinus</i>	<i>C. atrovirens</i>	<i>C. dibaphus</i>
<i>C. dibaphus</i>	<i>C. ionochlorus</i>	<i>C. sodagnitus</i>
Subsection <i>Glaucopodes</i>	<i>C. odoratus</i>	Section <i>Calochroi</i>
<i>C. anserinus</i> <sup>1</sup>	Subsection <i>Elegantiores</i>	Subsection <i>Arquati</i>
<i>C. glaucopus</i>	<i>C. claroflavus</i> <sup>8</sup>	<i>C. caesiocortinatus</i>
Section <i>Caerulescentes</i>	<i>C. elegantior</i>	<i>C. calochrous</i> var. <i>coniferarum</i>
Subsection <i>Caerulescens</i>	Subsection <i>Percomes</i>	Subsection <i>Calochroi</i>
<i>C. boudieri</i>	<i>C. mussivus</i>	<i>C. calochrous</i>
<i>C. caerulescens</i>	<i>C. nanceiensis</i>	<i>C. citrinolilacinus</i>
<i>C. dionysae</i>	Subsection <i>Rufoolivacei</i>	Section <i>Claricolores</i>
<i>C. sodagnitus</i>	<i>C. cupreorufus</i>	Series <i>Turmalis</i>
Subsection <i>Cumatilis</i>	<i>C. odorifer</i>	<i>C. turmalis</i>
<i>C. cumatilis</i>	<i>C. prasinus</i>	Subsection <i>Violaceipedes</i>
<i>C. praestans</i>	<i>C. rufoolivaceus</i>	<i>C. provencalis</i>
Subsection <i>Variicolores</i>	<i>C. xanthophyllus</i>	Section <i>Delibuti</i>
<i>C. balteatocumatilis</i>	Subsection <i>Splendentes</i>	Subsection <i>Delibuti</i>
<i>C. variicolor</i>	<i>C. citrinus</i>	<i>C. subtortus</i>
<i>C. varius</i>	<i>C. fulvocitrinus</i> <sup>9</sup>	Section <i>Fulgentes</i>
Section <i>Fulvi</i>	<i>C. meinhardii</i> <sup>10</sup>	Subsection <i>Elegantiores</i>
<i>C. cereifolius</i>	<i>C. splendens</i> <sup>11</sup>	<i>C. elegantior</i>
<i>C. elegantior</i>	Subsection <i>Sulfurini</i>	<i>C. pseudofulmineus</i>
<i>C. pseudofulmineus</i>	<i>C. flavovirens</i>	Section <i>Glaucopodes</i>
Section <i>Phlegmacium</i>	Section <i>Glaucopodes</i>	Subsection <i>Glaucopodes</i>
Subsection <i>Multiformes</i>	<i>C. anserinus</i>	<i>C. glaucopus</i>
<i>C. lustrates</i>	<i>C. dionysae</i>	Subsection <i>Magici</i>
<i>C. multiformis</i>	<i>C. glaucopus</i>	<i>C. dionysae</i>
<i>C. osmophorus</i>	Section <i>Infracti</i>	Section <i>Laeticolores</i>
<i>C. pseudonapus</i>	<i>C. infractus</i>	Subsection <i>Laeticolores</i>
Subsection <i>Phlegmacium</i>	Section <i>Multiformes</i>	<i>C. claroflavus</i>
<i>C. saginus</i> <sup>2</sup>	<i>C. cumatilis</i>	<i>C. cupreorufus</i>
<i>C. turmalis</i>	<i>C. multiformis</i>	<i>C. flavovirens</i>
<i>C. vulpinus</i> <sup>3</sup>	<i>C. praestans</i>	<i>C. odorifer</i>
Section <i>Sauri</i> (= <i>Laeticolores</i> )	<i>C. turmalis</i>	<i>C. prasinus</i>
Subsection <i>Orichalcei</i>	Section <i>Phlegmacium</i>	<i>C. rufoolivaceus</i>
<i>C. atrovirens</i>	Subsection <i>Triumphantes</i>	<i>C. xanthophyllus</i>
<i>C. citrinus</i>	<i>C. saginus</i>	Subsection <i>Percomes</i>
<i>C. claroflavus</i>	<i>C. triumphans</i>	<i>C. mussivus</i> <sup>12</sup>
<i>C. cupreorufus</i> <sup>4</sup>	<i>C. variiformis</i>	<i>C. nanceiensis</i>
<i>C. flavovirens</i> <sup>5</sup>	<i>C. varius</i>	Subsection <i>Splendentes</i>
<i>C. ionochlorus</i>	Subsection <i>Vulpini</i>	<i>C. atrovirens</i>
<i>C. meinhardii</i> <sup>6</sup>	<i>C. vulpinus</i>	<i>C. citrinus</i>
<i>C. odoratus</i>	Section <i>Phlegmacioides</i>	<i>C. meinhardii</i> <sup>3</sup>
<i>C. odorifer</i>	Subsection <i>Balteati</i>	<i>C. splendens</i> <sup>14</sup>
<i>C. prasinus</i>	<i>C. balteatocumatilis</i>	Section <i>Multiformes</i>
<i>C. rufoolivaceus</i>	Subsection <i>Variicolores</i>	Subsection <i>Multiformes</i>
<i>C. splendens</i>	<i>C. coalescens</i>	<i>C. multiformes</i>

TABLE II. Continued

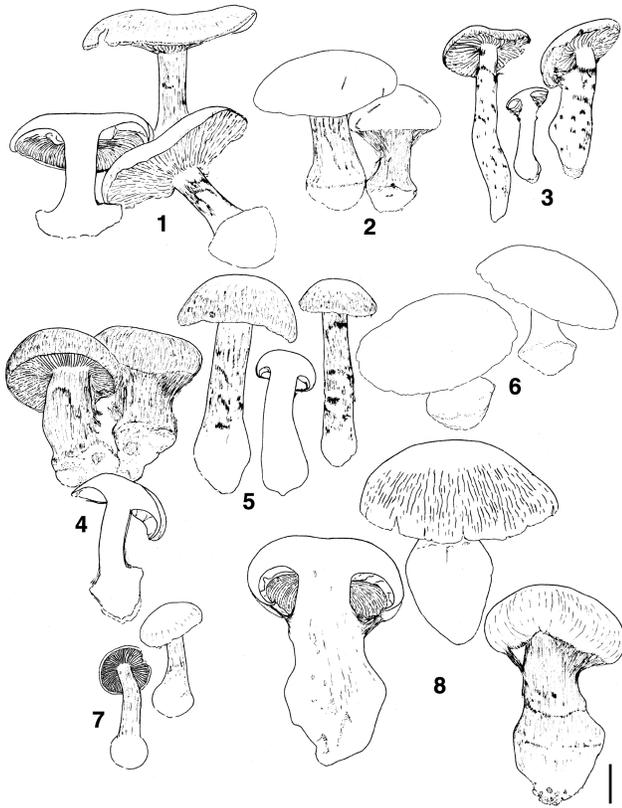
Moser (1960, 1983, 1986)	Brandrud et al (1990–1998), Brandrud 1996a, b, 1998a, b.	Moëgne-Loccoz et al (1990–2001)
<i>C. xanthophyllus</i>	<i>C. variicolor</i>	Section <i>Patibiles</i>
Subsection <i>Percomes</i>	Section <i>Scauri</i>	Subsection <i>Balteati</i>
<i>C. mussivus</i> <sup>7</sup>	<i>C. porphyropus</i>	<i>C. balteatocumatilis</i>
<i>C. nanceiensis</i>	<i>C. scaurus</i>	Subsection <i>Cyanipedes</i>
Subsection <i>Purpurascences</i>		<i>C. anserinus</i>
<i>C. porphyropus</i>		Subsection <i>Patibiles</i>
<i>C. purpurascens</i>		<i>C. coalescens</i>
<i>C. scaurus</i>		<i>C. variicolor</i>
<i>C. viridocaeruleus</i>		Section <i>Phlegmacium</i>
Section <i>Triumphantes</i>		Subsection <i>Ophiopodes</i>
<i>C. cephalixus</i>		<i>C. vulpinus</i>
<i>C. triumphans</i>		Subsection <i>Phlegmacium</i>
		<i>C. saginus</i>
		Subsection <i>Triumphantes</i>
		<i>C. cephalixus</i>
		<i>C. triumphans</i>
		Series <i>Varius</i>
		<i>C. variiformis</i>
		<i>C. varius</i>
		Section <i>Thalliophili</i>
		Subsection <i>Infracti</i>
		<i>C. infractus</i>
		Subsection <i>Purpurascences</i>
		<i>C. porphyropus</i>
		<i>C. purpurascens</i>
		Subsection <i>Thalliophili</i>
		<i>C. scaurus</i>

<sup>1</sup> *C. amoenolens* R. Hry. ex P. D. Orton, <sup>2</sup> *C. subvalidus* R. Hry., <sup>3</sup> *C. rufoalbus* Kühner, <sup>4</sup> *C. orichalceus* Fr., <sup>5</sup> *Phlegmacium olivellum* (R. Hry.) M. M. Moser ss. M. M. Moser, <sup>6</sup> *C. vitellinus* M. M. Moser, <sup>7</sup> *C. russeoides* M. M. Moser, <sup>8</sup> *C. humolens* Brandr., <sup>9</sup> *Phlegmacium pseudosulphureum* f. *pantherinum* Schaeff., <sup>10, 13</sup> *C. splendens* R. Hry. ssp. *meinhardii* (Bon) Brandr. & Melot, <sup>11, 14</sup> *C. splendens* R. Hry. ssp. *splendens*, <sup>12</sup> *C. russeus* R. Hry.

slightly gelatinous (viscid) to gelatinous. Hyphae of the gelatinous layer originate from the universal veil and the upper stratum of the epicutis. In *C. variicolor*, *C. coalescens* and *C. balteatocumatilis*, the poorly developed gelatinous layer was composed of relatively few hyphae; intermediate development of the gelatinous layer was observed in *C. caerulescens*, *C. glaucopus*, *C. pseudonapus*, *C. saginus*, *C. triumphans*, *C. variiformis* and *C. varius*, whereas in the remaining analyzed taxa the gelatinous layer was composed of various hyphal strata. The hyphae involved were relatively thin, cylindrical, hyaline to pigmented, sometimes weakly zebra-striped or with granular epiparietal incrustations and embedded in a matrix. The orientation of the hyphae in the deeper part was nearly parallel to the basal epicutis and becoming irregularly ascending toward the outer part. The hyphae of the universal veil on the pileus surface were narrow, hyaline to slightly pigmented.

*Microscopical characters.*—*Pileipellis*. The distribution of this character in the studied *Phlegmacium* species is given in FIG. 19. We recognized two pileipellis types: a “pileipellis simplex” consisting only of an epicutis (FIG. 9) and a “pileipellis duplex” consisting of an external epicutis and an internal hypocutis (FIG. 10). Intermediate forms were observed in *C. variicolor*. The epicutis consisted of an upper zone, forming part of the gelatinous layer with subparallel to ascendent sparse hyphae, and a basal zone, formed by densely arranged hyphae in subradial arrangement. In older basidiomes, the hyphae of the basal part of the epicutis sometimes become faintly striped or pigmented with punctate epiparietal incrustations. The hypocutis was characterized by ovoid, ellipsoid to subglobose, hyaline to pigmented hyphal segments, which sometimes were embedded in a colored amber-like matrix.

*Microscopical characters.*—*Context*. The pileus context consists of hyaline to pigmented, narrow or inflated



FIGS. 1–8. Basidiome habits of some European *Phlegmacium* species. 1. *Cortinarius multififormis*. 2. *C. elegantior*. 3. *C. vulpinus*. 4. *C. glaucopus*. 5. *C. saginus*. 6. *C. osmophorus*. 7. *C. scaurus*. 8. *C. praestans*. Scale bar = 2 cm.

hyphae. Inflated hyphae were predominant toward the lower layers of the context. In addition, cylindrical to tortuous oleiferous hyphae were observed in the pileus context.

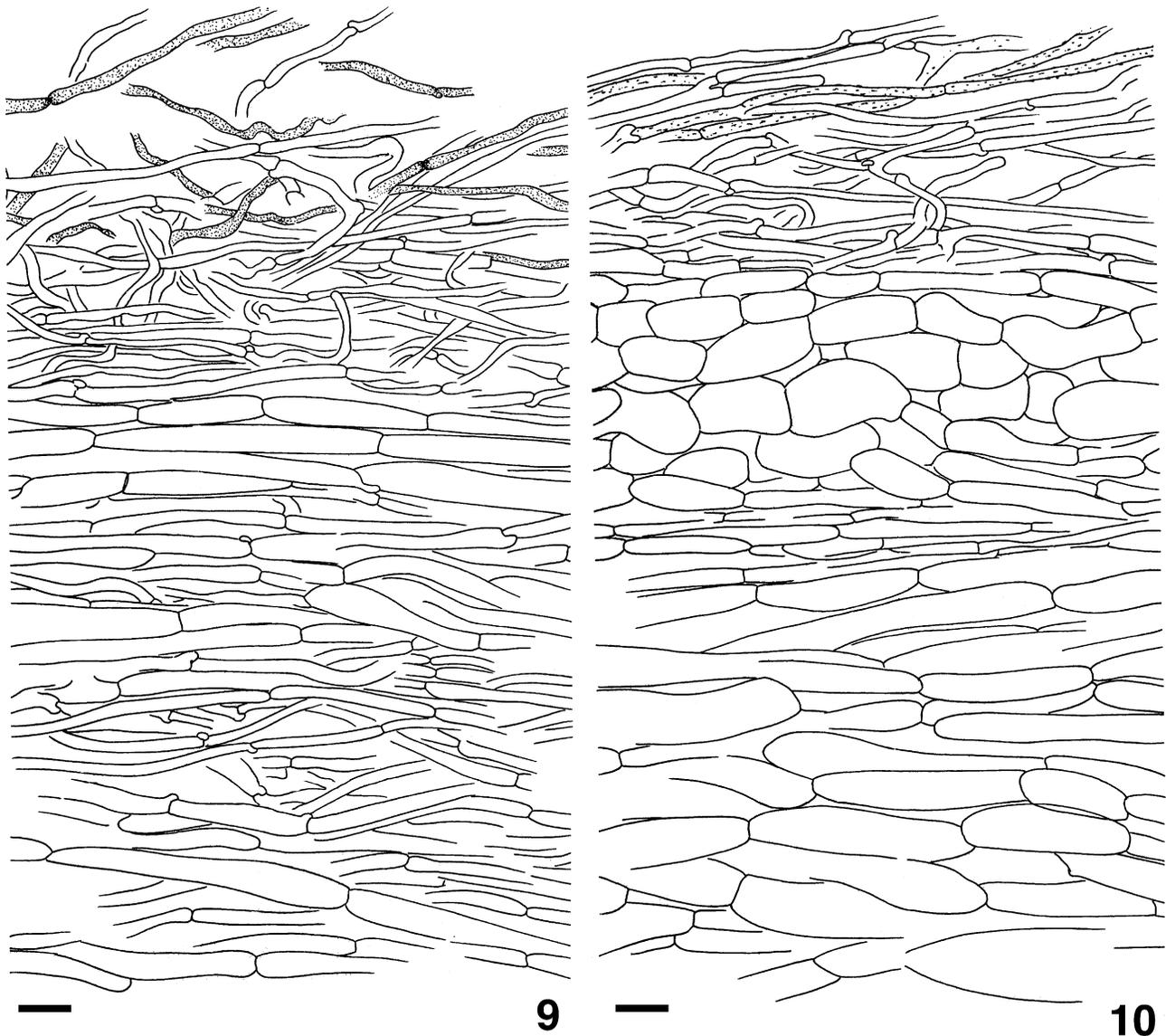
**Microscopical characters.—Lamellar structure.** The lamellar trama was regular, consisting of a lateral stratum of long, cylindrical, relatively narrow, parallel to subparallel, hyaline to pigmented hyphae, and a mediostratum with long, cylindrical to relatively short and inflated hyphal segments in parallel arrangement, especially toward the pileus context. Old basidia with wine-red content were present in *C. cereifolius*, *C. claroflavus*, *C. elegantior*, *C. flavovirens*, *C. fulvocitrinus*, *C. nanceiensis*, *C. mussivus*, *C. splendens* and *C. meinhardii*; basidia with purple content were found in *C. cupreorufus*, *C. odorifer*, *C. prasinus*, *C. rufolivaceus* and *C. xanthophyllus*. Lageniform pleuro- and cheilocystidia, with epiparietal incrustations in fresh material characterize *C. subtortus*. Abundant cylindrical to tortuous sterile hyphal elements were observed on the lamellar edges of *C. arcuatorum*, *C. dibaphus* and *C. prasinus*.

**Microscopical characters.—Basidiospores.** FIGURES 11–18 show the main types of basidiospore shape in the studied *Phlegmacium* spp.; an overview is given in FIG. 19. Basidiospores most frequently were citriform, followed by amygdaliform shape; few taxa possessed subfusoid or subglobose basidiospores. An infraspecific variation of the basidiospores ranging from amygdaliform to slightly citriform (= subcitriform) was found in various taxa. Most *Phlegmacia* studied were characterized by moderately to strongly ornamented basidiospores, with the exception of *C. cumatilis*, *C. lustratus* and *C. turmalis*, in which the basidiospores were scarcely ornamented.

**Microscopical characters.—Hyphal coloration in 3% KOH.** Most external hyphae of the gelatinous layer turned dark-olive with a gray tone when treated with 3% KOH in *C. atrovirens*, *C. ionochlorus*, *C. odoratus*, *C. splendens* and *C. meinhardii*, whereas they became yellowish-green in *C. cephalixus*, *C. nanceiensis*, *C. mussivus*, *C. prasinus*, *C. scaurus* and *C. xanthophyllus* and pink in *C. arcuatorum* and *C. sodagnitus*. Yellowish-golden to yellowish-brown coloration of the epicutis and hypocutis characterized *C. balteatocumatilis*, *C. caeruleascens*, *C. cephalixus*, *C. coalescens*, *C. pseudonapus*, *C. saginus*, *C. triumphans*, *C. varicolor*, *C. variiformis*, *C. varius* and *C. vulpinus*. In *C. arcuatorum* and *C. dibaphus*, the epicutis and context turned pink, and in various taxa a wine-red reaction was observed (see FIG. 19).

**Microscopical characters.—Phylogenetic analysis.** Results from the Bayesian molecular phylogenetic analysis are illustrated in FIG. 19. The groupings obtained from phylogenetic analysis showed many correlations with current classification concepts for species of *Phlegmacium* in Europe (Moser 1960, 1983, 1986; Brandrud et al 1990–1998; Bidaud et al 1994; Brandrud 1996a, b, 1998a; Moënne-Loccoz et al 1990–2001). The sections proposed by Brandrud et al (1990–1998) and Brandrud (1996a, b, 1998a) show an especially high degree of congruence with subgroups detected in our analyses. Among others, these subgroups are supported by our molecular analysis:

The species assigned to section *Fulvi* were distributed in two clusters. The main cluster (FIG. 19, top), containing most species of section *Fulvi*, was divided into two subgroups; one of these is formed by the species *C. arcuatorum*, *C. dibaphus*, *C. pseudofulmineus*, *C. elegantior*, *C. meinhardii*, *C. odoratus*, *C. splendens*, *C. ionochlorus*, *C. atrovirens* and *C. cereifolius*. The species *C. arcuatorum* and *C. dibaphus* (the latter included in section *Calochroi* in the Brandrud system) consistently were placed within section *Fulvi* as neighbors to *C. pseudofulmineus*. Molecular analysis significantly supported subsection *Atrovirentes* within



FIGS. 9–10. Pileipellis anatomy. 9. Longitudinal section of the pileus of *Cortinarius calochrous* (pileipellis simplex) showing gelatinous layer, epicutis, and context. 10. Longitudinal section of the pileus of *C. viridocaeruleus* (pileipellis duplex) showing viscid layer, epicutis, hypocutis, and context. Scale bar = 20  $\mu\text{m}$ .

section *Fulvi*, containing the species *C. odoratus*, *C. ionochlorus* and *C. atrovirens*. The species pair *C. meinhardii* and *C. splendens* were included in this subsection. The remaining species belonging to the *Fulvi* main group, *C. citrinus*, *C. xanthophyllus*, *C. prasinus*, *C. multififormis* ss. M. M. Moser, *C. rufolivaceus*, *C. odorifer*, *C. cupreorufus*, *C. flavovirens* and *C. claro-flavus* appear basal to those discussed above; their phylogenetic relationships were unresolved in our analysis. A second cluster, including the species *C. nanceiensis* and *C. mussivus*, was placed outside the *Fulvi* main group, close to *C. cephalixus*. The species *C. fulvocitrinus* was placed in section *Calochroi*.

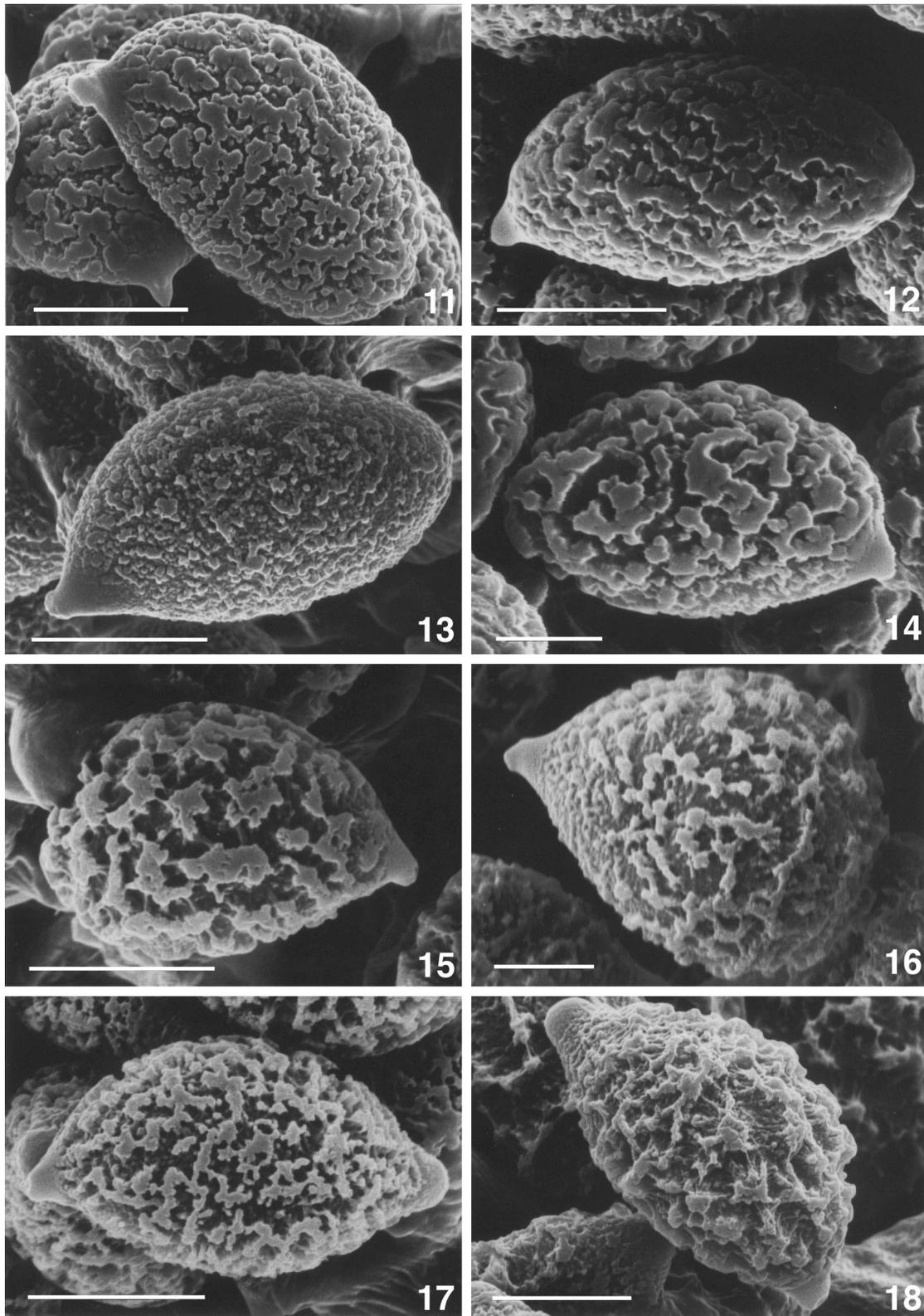
Section *Calochroi*, including the species *C. calo-*

*chrous*, *C. calochrous* var. *coniferarum*, *C. sodagnitus* and *C. citrinolilacinus*. The species *C. osmophorus* and *C. fulvocitrinus* also were assigned to this group in our analysis.

Section *Glaucopodes*, containing the species *C. anserinus*, *C. dionysae* and *C. glaucopus*. The taxa *C. viridocaeruleus* and *C. caerulescens* also were included in this section.

Section *Phlegmacium* containing the species *C. saginus*, *C. vulpinus*, *C. varius*, *C. triumphans* and *C. variiformis*. The species *C. saginus* and *C. vulpinus* were significantly grouped together.

Section *Phlegmacioides*, including the species *C. variicolor*, *C. coalescens* and *C. balteatocumatilis*; the



FIGS. 11–18. Some principal basidiospore shapes in European *Phlegmacium* species. Ellipsoid. 11. *Cortinarius scaurus*. Ellipsoid-amygdaliform. 12. *C. vulpinus*. Amygdaliform. 13. *C. cephalixus*. Ellipsoid-amygdaliform. 14. *C. purpurascens*. Subglobose. 15. *C. caesiocortinatus*. 16. *C. infractus*. Citriform. 17. *C. anserinus*. 18. *C. elegantior*. Scale bars: 11–13 = 4  $\mu\text{m}$ , 14 = 2  $\mu\text{m}$ , 15 = 4  $\mu\text{m}$ , 16 = 2  $\mu\text{m}$ , 17–18 = 4  $\mu\text{m}$ .

species *C. coalescens* and *C. variicolor* were grouped together.

Section *Scauri*, including the species *C. scaurus*, *C. porphyropus* and *C. purpurascens*; *C. porphyropus* and *C. purpurascens* appear as sister taxa.

#### DISCUSSION

In this section we discuss our results, comparing our molecular phylogenetic hypotheses with the distribution of pigment contents and macro- and microscopical characters of the basidiomes. According to the congruence of our molecular phylogenetic results and the classification system proposed by Brandrud et al (1990–1998) and Brandrud (1996a, b, 1998a) for European Phlegmacia, we will base our remarks on this classification concept, although various species examined in this study have not yet been included in this system (see TABLE II).

*Section Fulvi*.—The main cluster containing most of the species of this section was supported by an a posteriori probability value of 80%, containing an even better-supported subgroup (100% a posteriori probability) that includes the species *C. arcuatorum*, *C. dibaphus*, *C. pseudofulmineus*, *C. elegantior*, *C. meinhardii*, *C. odoratus*, *C. splendens*, *C. ionochlorus*, *C. atrovirens* and *C. cereifolius* (FIG. 19, top). The species *C. arcuatorum* and *C. dibaphus*, which are considered closely related in current classification systems (Moser 1960, 1983, 1986; Moënné-Locoz et al 1990–2001), are linked with an a posteriori probability of 100%. According to our results, *C. provencalis* occupies an isolated position and is placed basally to sections *Fulvi* and *Calochroi*.

Our phylogenetic analysis supports a close phylogenetic relationship between subsection *Atrovirentes* (*C. odoratus*, *C. ionochlorus* and *C. atrovirens*) and subsection *Splendentes* (*C. meinhardii* and *C. splendens*). A close relationship between *C. odoratus*, *C. ionochlorus* and *C. atrovirens* grouped in subsection *Atrovirentes* first was proposed by Oertel (1984) and Oertel and Steglich (1985), who detected atrovirin and flavomannin as main pigments, while a 4-hydroxy-flavomannin-6,6'-dimethylether pigment was detected in *C. meinhardii* and *C. splendens* (subsection *Splendentes*). Moreover, we have observed a similar KOH reaction of the outermost external hyphae of the gelatinous layer in the members of both subsections. A distinctive character of members of subsection *Atrovirentes* is that the basidiomes become black pigmented when dried (Steglich and Oertel 1985). The specimens of *C. ionochlorus* and *C. atrovirens*, traditionally distinguished by the coloration of the lamellae and ecology, showed identical DNA se-

quences. The differences in the lamellar coloration probably are caused by light-sensitive pigments under different ecological conditions: Basidiomes of *C. ionochlorus*, which are associated with frondose trees, grow under a layer of fallen leaves in their early developmental stage and thus are shielded from sunlight. Conversely, the basidiomes of *C. atrovirens*, which are affected early by sunlight, thus might become strongly pigmented.

Phylogenetic relationships among *C. citrinus*, *C. xanthophyllus*, *C. prasinus*, *C. multiformis*, *C. rufolivaceus*, *C. odorifer*, *C. cupreorufus*, *C. flavovirens* and *C. claroflavus* remained unresolved in our molecular phylogenetic analysis due to very similar sequences in the studied rDNA regions. This is congruent with the uniformity of microscopic structures that characterize these species. *Cortinarius nanceiensis* and *C. mussivus* were placed together but separate from the remaining members of section *Fulvi*. Both taxa are well characterized by a cylindrical to clavate stipe, a character not present in the remaining taxa of section *Fulvi* that we examined. Similarities in pigment contents—phlegmacin-8'-methylether, which has been considered as a derived character in this group (Oertel 1984, Steglich and Oertel 1985, Brandrud 1998b)—support the close relationship between *C. nanceiensis* and *C. mussivus*. These two species clustered with *C. cephalixus* in our molecular phylogenetic analysis (95% a posteriori probability), which is consistent with similarities in stipe shape, pileipellis structure and probably in pigments of the basidiomes. *Cortinarius fulvocitrinus*, which contains flavomannin-6,6'-dimethylether (Oertel 1984, as *C. citrinus*), was classified in section *Fulvi* and subsection *Splendentes* by Brandrud (1998b). However, unlike other members of section *Fulvi*, the lamellae of *C. fulvocitrinus* are not yellow. Consistent with this observation, *C. fulvocitrinus* was separated from section *Fulvi* and placed among members of section *Calochroi* in our molecular analysis.

*Section Calochroi*.—The grouping of *C. calochrous*, *C. calochrous* var. *coniferarum*, *C. sodagnitus*, *C. citrinolacinus*, *C. osmophorus* and *C. fulvocitrinus* was supported by an a posteriori probability of 95%. The species *C. osmophorus* was ascribed to section *Triumphans* by Moser (1960, 1983, 1986), and *C. fulvocitrinus* was placed in section *Fulvi* (Brandrud 1998b; see discussion above). Similarities in habit concerning the stipe shape, flesh coloration (except *C. fulvocitrinus*), pileipellis structure (pileipellis simplex), degree of development of the gelatinous layer and basidiospore morphology (except *C. sodagnitus*) can be correlated with our molecular grouping. While yellow to yellowish-brown colors of the pileus character-



FIG. 19. Bayesian inference of phylogenetic relationships in European *Phlegmacium* species: Markov chain Monte Carlo analysis of an alignment of nuclear rDNA sequences from the ITS region (including the gene coding for the 5.8S ribosomal subunit) and the D1/D2 region of the large ribosomal subunit, with the general time-reversible model of DNA substitution with gamma-distributed substitution rates (GTR+G), random starting trees, default starting parameters of the substitution model and involving four incrementally heated Markov chains. Majority rule consensus tree from 18 000 trees sampled after

ize all the species in this section, further studies of pigment contents might help to clarify the delimitation of this group. The analyzed sequences of *C. calochrous* and *C. citrinolilacinus* were identical, reflecting the poor morphological differences on which the separation of both species is based (Moser 1960).

**Section Glaucopodes.**—Section *Glaucopodes*, containing the species *C. anserinus*, *C. dionysae*, *C. glaucopus* with the incorporation of *C. viridocaeruleus* and *C. caeruleus*, was supported as a monophyletic group by an a posteriori probability of 96%. In classical systems (Moser 1960, 1983, 1986; Brandrud et al 1990–1998; Moënne-Loccoz et al 1990–2001), the latter two species were classified in different sections (TABLE II). Macroscopical characters that correlate with the grouping in our analysis are similarities of basidiome coloration and stipe shape. At the microscopic level, all members in this group present a similar pileipellis structure with a moderately developed gelatinous layer. The grouping of *C. viridocaeruleus* with *C. anserinus* and *C. dionysae*, which is present in our molecular analysis, is consistent with the basidiospore morphology of these species.

**Section Phlegmacium.**—The species *C. saginus*, *C. vulpinus*, *C. varius*, *C. triumphans* and *C. variiformis* were classified by Brandrud (1996a, b) in section *Phlegmacium*, which is supported by an a posteriori probability of 64% in our molecular analysis. Different systematic positions of these species have been proposed in the past (Moser 1960, 1983, 1986; Moënne-Loccoz et al 1990–2001). Members of section *Phlegmacium* are characterized by similarities of pileus and lamellar coloration, by a reduced gelatinous layer and by stipe shape. The presence of a veil in *C. saginus*, *C. triumphans* and *C. vulpinus* has been suggested as an adaptation to xerophilic conditions by Brandrud (1996a, b). At the microscopic level, the pileipellis morphology characterizes this group well; a similar observation was reported by Brandrud (1996a, b). *Cortinarius saginus* and *C. vulpinus* were grouped together with an a posteriori probability of 85% in our molecular analysis; the phylogenetic re-

lationships among the other members of this section remained unresolved.

**Section Phlegmacioides.**—The species of section *Phlegmacioides* (*C. variicolor*, *C. coalescens* and *C. balteatocumatilis*) included in our study were clustered together with an a posteriori probability of 92%. In traditional classification systems these species have been considered closely related (Moser 1960, 1983, 1986; Brandrud et al 1990–1998; Brandrud 1998a; Moënne-Loccoz et al 1990–2001). Macromorphological traits consistent with this association are the texture and consistency of the pileus surface, the stipe shape and basidiome coloration. The violet pigments turn brown with the age of the basidiomes (Brandrud 1998a). The flesh becomes yellow with KOH. At the microscopic level, members of this section have a poorly developed gelatinous layer and hypocutis. Though grouped together with an a posteriori probability of 100%, *C. variicolor* and *C. coalescens* appear to represent different species according to their genetic distance. Brandrud (1998a) differentiated them by ecology, odor and coloration of the basidiomes.

**Section Multifformes.**—The close phylogenetic relationship between *C. praestans* and *C. cumatilis* was strongly supported by an a posteriori probability of 98% in our molecular analysis. This agrees with classical taxonomy, in which both species also have been treated as closely related. The basidiomes of both species show similar coloration and differ in their basidiospore morphology. In our molecular analysis, *C. multiformis* ss. M.M. Moser was separated from these species and grouped among members of section *Fulvii*; this is justified because of stipe shape and probably of basidiome pigments. There is some taxonomic confusion regarding the latter taxon: It is possible that *C. multiformis* ss. Brandrud, which is not included in the present molecular study and which differs from *C. multiformis* ss. M.M. Moser in habit and ecology, represents a different species that is phylogenetically closer to *C. praestans* and *C. cumatilis*.

←

convergence to stationarity; the topology was rooted with *Laccaria amethystina*. Numbers on branches are estimates for a posteriori probabilities. Branch lengths are maximum-likelihood estimates and are scaled in terms of expected numbers of nucleotide substitutions per site.

Characters indicated; I) pileipellis duplex present (●) or absent (○), II) basidiospore shape: a = amygdaliform, ac = amygdaliform to slightly citriform, c = citriform, e = ellipsoid, ea = ellipsoid-amygdaliform, s = subglobose and sf = subfusoid, III) bulbous stipe (submarginate to marginate) present (●) or absent (○), IV) color of basidiome flesh (context) in fresh material: v = with violet shade, w = white (sometimes slightly other) and y = yellow to green, V) wine-red microscopical reaction with 3% KOH on pileus sections (and sometimes on lamellar trama) present (●) or absent (○).

*Section Infracti*.—The DNA sequences of three collections of *C. infractus* showed a certain variability. In tree topology, *C. infractus* is related to *C. subtortus*, with an a posteriori probability of 96%. However, *C. subtortus* also was placed in the group with *C. cephalixus*, *C. nancaiensis* and *C. mussivus* in some re-iterations of the MCMC analysis (data not shown). Moser (1960, 1983, 1986) included *C. subtortus* and *C. infractus* in section *Amarescentes*, based on their lamellar coloration, a bitter taste and basidiospore morphology. *Cortinarius subtortus* is distinguished by the presence of cystidia and *C. infractus* by indole alkaloid content (Steglich et al 1984).

*Section Scauri*.—The group formed by *C. scaurus*, *C. porphyropus* and *C. purpurascens* was weakly supported by an a posteriori probability of 56% in the molecular phylogenetic analysis; *C. porphyropus* and *C. purpurascens* were linked with an a posteriori probability of 100%. Moser (1960, 1983, 1986) classified these species in Section *Laeticolores*, subsection *Purpurascens*, and Moënne-Loccoz et al (1990–2001) in section *Thalliophili*, subsection *Purpurascens* (*C. porphyropus* and *C. purpurascens*) and subsection *Thalliophili* (*C. scaurus*). *Cortinarius scaurus*, *C. porphyropus* and *C. purpurascens* appear in a basal position on the tree (FIG. 19) and separate from the main group of subgenus *Phlegmacium*. Although the clade is weakly supported, characters that justify it include a positive lugol reaction and the change of basidiome coloration when touched or scratched.

*ITS sequence variability*.—ITS sequences in general show little variation at the species level independent of geographical origin. However, in *C. glaucopus*, *C. infractus* and *C. scaurus*, some variation was found among specimens, which might reflect the presence of subspecies or even different (but morphologically very similar) species. Based on macroscopical characters, various subspecies have been recognized in Europe (Moser 1960, Brandrud et al 1990–1998).

Various species with particular morphological, and probably chemical characters, occupied isolated positions in the molecular analysis. These species will have to await the inclusion of a broader spectrum of *Cortinarius* species in molecular analyses before more meaningful hypotheses about their phylogenetic positions can be derived.

*Phylogenetic relationships between sections*.—Our molecular analyses indicate a close relationship between sections *Fulvi* (excluding *C. mussivus* and *C. nancaiensis*) and *Calochroi*; they were supported as sister groups with an a posteriori probability of 96%. Representatives of both sections are distinguished by a tendency toward citriform basidiospores, a well-

developed gelatinous layer and epicutis, a pileipellis simplex, brightly colored basidiomes and by a marginated, bulbous stipe. Reijnders (1979, 1986) correlated a marginate stipe shape with a pileocarpous development in *Phlegmacium* species, i.e., a development where the pileus differentiates before the stipe expands. This type of development was considered a derived character by Singer (1986); it might be more efficient in preventing desiccation in early development of basidiomes. In our phylogenetic tree (FIG. 19), sections *Fulvi* and *Calochroi* appear separated from the remaining taxa by a relatively large genetic distance. Other possibly derived characters that might be used to confirm this grouping are a well-developed gelatinous layer and epicutis that protect the basidiomes against the rain. Regarding ecology, members of sections *Fulvi* and *Calochroi* grow preferentially on basic soil while species of the remaining sections that are included in our study were restricted to acidic soils.

A close relation between sections *Glaucopodes* and *Caerulescentes* (*C. caerulescens*) as reflected in the molecular analysis could be correlated with the micromorphology of the pileipellis (duplex) and gelatinous layer and also with basidiome coloration and stipe shape. However, more species of section *Caerulescentes* must be included in further analyses to clarify the status of this section. Similarities of stipe shape among species of sections *Calochroi*, *Fulvi*, *Glaucopodes* and *Caerulescentes* are consistent with the close phylogenetic relationship among these sections that is suggested by our molecular analysis.

Results from molecular and morphological analyses suggest the need to revise the current classification systems for subgenus *Phlegmacium* in Europe. However, at this time, it would be premature to propose a new classification concept. Other important *Phlegmacium* species will have to be included in future analyses, and comprehensive morphological, chemical and molecular analyses covering the whole systematic spectrum of *Cortinarius* species will have to be performed before sound classification concepts can be erected, which very probably no longer will address *Phlegmacium* as a subgenus but will deal with its present sections in various new phylogenetic subgroups of the fascinating genus *Cortinarius*.

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