Phylogenetic relationships in Cortinarius with focus on North European species

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Cortinarius is an ectomycorrhizal Agaricales genus with high diversity of which rDNA sequences of 86 species together with four outgroup taxa were investigated phylogenetically by aid of Maximum Likelihood and Bayesian analyses. The Cortinarius data set represents 81 taxa from the Northern Hemisphere showing the main variation spectrum among the species. In addition, five species from the Southern Hemisphere are included. The phylogenetic tree of Cortinarius gives statistical support to twelve monophyletic groups in the upper level. They are discussed in context of morphology, chemistry (secondary compounds), and ecology. The phylogenetic tree lacks, however, satisfactory support for its backbone. Several species could not be included in any group, especially those forming the basal framework of the tree. Of special interest is a “superclade” comprising eight of our monophyletic clades and two singletons. Here we find the majority of species with soluble pigments of octaketide origin, all species with compounds of nonaketide origin, the majority of species with hygrophanous pileus, few species with viscid pileus, and no species with bulbous stipe base. Moreover, all species except one have duplex pileus cuticle. The morphological traits are not indicative for any clade, although some are more frequent in some clades than others. During the evolution they have been gained and lost several times. The chemical characteristics are – to a certain degree – more indicative for the clades. The evolution and ecological role of these compounds are discussed. Concerning the North European species, there are ecological differences between the clades, especially between clades specializing to rich or calcareous forests and clades specializing to poor forests or arctic-alpine environments.

Key words: Cortinarius, phylogeny, morphology, secondary compounds, ecology

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Introduction

Cortinarius (Pers.) Gray is a genus of Agaricales (Basidiomycota) characterised by a fugacious veil enveloping the basidiocarp, and a cortina at first covering the lamellae, but later vanishing in expanding basidiocarps. The basidiocarp size, shape, and colour vary considerably among species. The lamellae are emarginate to adnate, variously coloured, soon becoming brownish from mature, brown-pigmented basidiospores. The basidiospores are usually verrucose, highly variable
in size, and the colour may vary from pale ochre fulvous to rusty brown.

Diversity within the *Cortinarius* is high; over 2000 species are referred to the genus (Brandrud et al. 1990-2013). *Cortinarius* spp. are important ectomycorrhiza formers (Kärén et al. 1996) in temperate deciduous and boreal coniferous forests and arctic and alpine tundra (Ammirati & Smith 1978, Moser 1983, Gulden & Torkelsen 1996, Brandrud et al. 1990-2013) but do occur in tropical areas (Peintner et al. 2003) or Mediterranean evergreen thickets (Suáres-Santiago et al. 2009). The genus is also common and widespread in the Southern hemisphere (Moser & Horak 1975, Keller et al. 1988, Chambers et al. 1999, Garnica et al. 2001). Interesting alkaloids or (R)-β-dopa derivatives are found in *C. infractus* (Pers.) Fr., *C. subtortus* (Pers.) Fr., and *C. violaceus* (L.) Gray (von Nussbaum et al. 1998, Spiteller et al. 2000, Brondz et al. 2007, Brondz & Høiland 2008, Teichert et al. 2008). All species of the infrageneric group *Dermocybe* (Fr.) Trog and some species belonging to the groups *Telamonia* (Fr.) Trog. *Phelegmacium* (Fr.) Trog (the latter in the traditional circumscription (Brandrud et al. 1990-2013)) contain vividly coloured anthraquinones or pre-anthraquinones belonging to the octaketides (Gill & Steglich 1987). *Cortinarius venetus* (Fr.) Fr., *C. cotoneus* Fr., and *C. colymbadinus* Fr. contain yellow-green fluorescent xanthones and anthraquinones belonging to the nonaketides or octaketides (Gill & Steglich 1987).

Traditionally *Cortinarius* has been divided into several infrageneric groups according to various authors (see Garnica et al. (2005) for a nice overview). The traditional subgenera included in this paper follow the treatment by Moser (1983) and Moser & Horak (1975): *Cortinarius, Dermocybe* (by us at subgenus level), *Leprocype* M.M. Moser, *Myxacium* (Fr.) Trog, *Phelegmacium, Sericeocybe* P.D. Orton, and *Telamonia*. The aforementioned traditional classifications are only partly support-ed by molecular phylogenetic studies (Hoiland & Holst-Jensen 2000, Peintner et al. 2003, Peintner et al. 2004, Garnica et al. 2005, Harrower et al. 2011, Ryberg & Matheny 2011). *Cortinarius violaceus*, the type species of the genus (Clements & Shear 1931), belongs to subgenus *Cortinarius* in the traditional classification. This subgenus has only ten global species (Peintner et al. 2004) and is characterised by basidiocarps with dry, squamulose pileus surface, lamellae with cheilo- and pleurocystidia, and spores with a distinct suprahilar smooth spot, a plage. The peculiar violet iron complex is unique for *C. violaceus* (von Nussbaum et al. 1998, Spiteller et al. 2000).

The monophyly of *Telamonia* is supported by several authors (Liu et al. 1995, Chambers et al. 1999, Høiland & Holst-Jensen 2000, Peintner et al. 2003, Peintner et al. 2004, Garnica et al. 2005, Harrower et al. 2011), only *Acetosi, Camphorati, Fulvescentes, Illumini, Laeti, Obtusi, and Renidentes* are not included based on molecular studies (Niskanen 2008). The name *Telamonia* is vindicated since the clade includes the type species, *C. torvus* (Fr.) Fr. (Singer & Smith 1946), of this subgenus (Garnica et al. 2005). The traditional subgenus *Sericeocybe* (Orton 1958) is, however, engulfed in clade *Telamonia* (Peintner et al. 2004, Garnica et al. 2005), since it contains the type species of the former: *C. alboviolaceus* (Pers.) Fr. (Singer 1986). A sister relationship between *C. brunneus* (Pers.) Fr. and *C. gentilis* (Fr.) Fr. has previously been documented (Hoiland & Holst-Jensen 2000, Niskanen et al. 2009), as well as between *C. alboviolaceus* and *C. armeniacus* (Schaeff.: Fr.) Fr. (Peintner et al. 2001).

*Dermocybe s. str.* (Høiland 1984), including the type, *C. cinnamomeus* (L.) Fr. (Singer 1986), is well supported (Liu et al. 1995, 1997; Chambers et al. 1999; Høiland & Holst-Jensen 2000; Peintner et al. 2004; Garnica et al. 2005; Harrower et al. 2011). Common for all species are pigments of octaketide origin (Høiland 1984, Gill & Steglich 1987). Two monophyletic lineages are identified in European and North American material, one *Dermocybe* line and one *Sanguinei* line, where *C. mali-corius* Fr. occupies a basal position (Niskanen et al. 2012, 2013b). However, the Southern Hemisphere species previously put in *Dermocybe* (Moser & Horak 1975) form clades outside this group: Splen-di (with *C. splendidus* (E. Horak) K. Griffiths and *C. kula* Grgurinovic) forms an own clade; *Icteri-
nula (with C. icterus (E. Horak) E. Horak and C. amoenus (M.M. Moser & E. Horak) G. Garnier) form a third entity; and Pauperae (with C. luteno-
striatus (M.M. Moser & E. Horak) E. Valenz. & G. Moreno, C. obscuroolivaceus (M.M. Moser)
Kuhn.-Fink. & Peintner, and C. flavificatus (E. Horak & M.M. Moser) E. Valenz & G. Moreno), a
fourth group (Garnica et al. 2003a, 2005; Peintner et al. 2004; Stefani et al. 2014).

The thin fleshy hygrophaneous, Telamonia-
like C. obtusus (Fr.) Fr. and C. acutus (Pers.) Fr. have repeatedly been demonstrated as a clade, Ob-
tusi, outside Telamonia (Høiland & Holst-Jensen
2000, Peintner et al. 2003, 2004; Garnica et al.

Clade Leprocybe comprises species with
strongly fluorescent nonaketide xanthones (Gill &
Steglich 1987). This clade represents the remains
of subgenus Phlegmacium (Peintner et al. 2004,
Harrower et al. 2011), since it contains C. cotoneus,
the type species (Singer 1986).

Clade Orellani comprises the toxigenic C.
orellanus and C. rubellus. Biosynthesis of the bi-
pyridines orellanine and orelline (Schumacher &
Høiland 1983, Gill & Steglich 1987) is a mono-
phyletic trait. Orellani was originally described as
a section of subgenus Leprocybe (Moser 1969),
but later elevated to a subgenus of its own (Gas-
parini 2004).

The subgenus Myxacium, as traditionally treat-
ed, has been divided into four sections: Myxacium,
Defibulati, Delibiti, and Vibratiles/Ochroleuci
(Høiland & Holst-Jensen 2000, (Seidl 2000, Peint-
ner et al. 2004, Garnica et al. 2005, Harrower et
al. 2011). Section Myxacium comprises the core of
this subgenus, including the type species, C. col-
limitus (Pers.) Fr. (Singer 1986), with Defibulati as
sister clade. The two other sections are not related
to these clades, and subgenus Myxacium with its
original inventory is polyphyletic.

Subgenus Phlegmacium in its traditional sense
appears para- or polyphyletic in molecular phy-
logenies (Høiland & Holst-Jensen 2000; Garnica
Calochroi forms a distinct and strongly supported
clade and comprises species with stout basidi-
ocarps, simple cuts, and emarginated bulb at stipe
base (Garnica et al. 2003b, 2005; Peintner et al.
2004; Frøslev et al. 2005, 2007); (Harrower et
al. 2011). Scauri/Purpurascences includes spe-
cies with violet-red reaction in KI3 (Høiland &
Holst-Jensen 2000; Garnica et al. 2003b, 2005;
Harrower et al. 2011). The aforementioned studies
also reveal several smaller, isolated clades. The
remaining part of the traditional subgenus Phleg-
macium constitutes a weakly supported group,
comprising several evolutionary lineages (Garnica
et al. 2005), e.g. Caerulescentes, Phlegmacioides,
Percomes, Phlegmacium, containing the type spe-
cies C. saginus (Fr.) Fr. (Singer 1986), and Prae-
tantes.

The genus Rozites P. Karst. is characterised by
having membranaceous partial veil forming a per-
sistent ring and a membranaceous universal veil.
It has previously been shown that Rozites should
be included within Cortinarius (Høiland & Holst-
2005, Harrower et al. 2011). It has been demon-
strated that Rozites is polyphyletic, suggesting that
a membranaceous veil has evolved in multiple in-
dependent events in Cortinarius (Peintner et al.
2002). Some fungi with sequestrate (gastroid or
partially closed) basidiocarps, have a polyphyletic
origin within genus Cortinarius (Peintner et al.
2001, Danks et al. 2010).

The aim of this study was to (1) evaluate the
evolutionary relationship in a molecular data set
of Cortinarius, including representative species
for the different subgenera and sections, perform-
ing phylogenetic analyses of the ribosomal gene
loci, containing the 18S, 5.8S, and 28S rDNA
genes and the internal transcribed spacer regions
(ITS1 and ITS2), and (2) trace infrageneric evo-
lation of macro- and micromorphological, chemi-
cal, and ecological characteristics in light of the
inferred molecular phylogeny, with focus on the
North European species.

Material and methods

Fungal material
The Cortinarius samples (Table 1) were collected in Nor-
way between 1985 and 2008. The sampling is covering the
main variation within North European species and is rang-
ing from arctic tundra to broadleaved temperate forests.
To give the correct name for clades, the type species for
the traditional subgenera and (some) sections are included
in the sampling (Clements & Shear 1931, Singer & Smith
In addition, some sequences (Table 1) were acquired from
the NCBI nr nucleotide database (http://www.ncbi.nlm.
in.gov/, as of 12.2012) to broaden our species sample to
include some species outside North Europe. Suitable out-
group species (Table 1) (Agrocybe praecox (PERS.) Fayod, Galerina stordalii A.H. Sm., Helvelona alpinum (J. Favre) Bruchet, and Phaseolusaglaia stagnina (FR.) Pegler & T.W.K. Young) were established based on previous molecular phylogenies of dark spored agarics including Cortinarius (Gülden et al. 2005). The nomenclature follows Index Fungorum (http://www.indexfungorum.org/Names/Names.asp, as of 12.2013). Dried, field-collected specimens are deposited in the fungal herbarium of the Natural History Museum, University of Oslo (O).

DNA extraction and PCR

DNA was obtained from dried specimens. Various protocols for DNA extraction were used: the 2% CTAB mini-prep method (Murray & Thompson 1980), the microwave miniprep procedure (Goodwin & Lee 1993) and the Dynabeads R DNA DirectTM System 1 extraction kit (Rudi et al. 1997). Primer pairs used in the PCR included PNS1/NS41 for the partial nr18S region, ITS5/ITS4 for the complete ITS1-5.8S-ITS2 region, and LR0R/LRS5 for the partial nr28S region (White et al. 1990). Template was amplified with PuReTaq Ready-To-Go PCR Beads (GE Healthcare). PCRs were run with the following cycling conditions: 1 x (3 min/ 94 °C), 40 x (30 s/ 94 °C, 30 s/ 50-52 °C, 1 min/ 72 °C), 1 x (10 min/ 72 °C).

PCR products were purified using ExoSAP-IT (Amersham Biosciences), prior to sequencing. Sequences were generated with an ABI 3730 high-throughput capillary electrophoresis sequencers using the PCR primers as sequencing primers.

Phylogenetic inference

The three rDNA genes (18S, 5.8S, and 28S) and the internal transcribed spacer regions (ITS1 and ITS2) were collectively aligned using MAFFT v6 Q-INS-I model (Hofacker et al. 2002, Kiryu et al. 2007, Katoh & Toh 2008), considering secondary RNA structure (default parameters used) as a criterion for the alignment. The alignment was checked manually using MacClade v4.07 (Maddison & Maddison 1992), before being inferred with Giblocks v0.91b (Cestresana 2000), under the least stringent parameters, to exclude poorly aligned positions and divergent regions from subsequent phylogenetic inferences. The inference of a concatenated rDNA alignment gave a 68% increase in informative nucleotide characters (825 to 491) compared to that of inferring just the 5.8S and the ITS regions. Ambiguous species were subsequently excluded from downstream analysis upon evaluation of the alignment and preliminary ML (RAxML) topologies. The dataset was analysed with MODLETEST (Posada & Crandall 1998) to establish the optimal model of nucleotide evolution; for all alignments the General Time Reversible (GTR) model was preferred for both the Akaike and Bayesian information Criterion (AIC and BIC). Maximum Likelihood (ML) analyses were performed with RAxML-VI-HPCv7.2.6, GTRCAT model with 25 rate categories (Stamatakis 2006). The most likely topology was established from 100 separate searches, and bootstrap analyses were performed with 500 pseudoreplicates. Bayesian analyses were carried out with MrBayes MPI version 3.1.2 (Huelsenbeck et al. 2001, Ronquist & Huelsenbeck 2003). Trees were generated from two independent runs with one heated and one cold chain in the Markov Chain Monte Carlo (MCMC) with 40,000,000 generations, sampling every 1000. Analyses ran until the average standard deviation of split frequencies was <0.01. Burn-in trees were set based on the assessment of likelihood plots and convergence diagnostics implemented in MrBayes. The Potential Scale Reduction Factor (PSRF) values for all inferences were ~1.0, indicating a good posterior probability distribution sample. The majority rule tree and posterior probabilities for each inference was constructed from a consensus of the sampled post burn-in trees. Species with an uncertain phylogenetic affinity (rogue taxa), were established using the RogueNaRok-algorithm (Aberer et al. 2013), with the following parameters; a majority-rule consensus threshold, support optimization and a Max dropset size of 5. Topological congruence between the inferred phylogenies was calculated using the d_2 index: http://max2.ese.u-psud.fr/bases/upresa/pages/devienne/index.html (de Vienne et al. 2007). All model estimation and phylogenetic analyses, unless otherwise stated, were done on the freely available Bioportal (Kumar et al. 2009) at the University of Oslo (http://www.bioportal.uio.no/, as of 12.2013).

Morphological, chemical, and ecological data


Results

Sequence amplification and assembly

205 DNA sequences from 69 Cortinarius species and four outgroup species were successfully amplified for the whole rDNA gene locus, except for C. gentilis, which failed for 28S, and C. torvus, which failed for 18S. The sequences are deposited in Genbank under the accession

Cortinarius croceus (Schaeff.) Gray D
Cortinarius cumatilis Fr. P
Cortinarius delibutus Fr. M
Cortinarius evernius (Pers.) Fr. T
Cortinarius flexipes (Fr.) Fr. T
Cortinarius gentilis (Fr.) Fr. T
Cortinarius glaucopus [Schaeff.] Fr. P
Cortinarius icterinus (Fr.) Fr. Horak D
Cortinarius idahoensis Ammirati & A.H. Sm. D
Cortinarius infractus (Pers.) Fr. P
Cortinarius longipes Fr. P
Cortinarius limonius (Fr.) Fr. L
Cortinarius luteostriatulus (M.M. Moser & E. Horak) E. Valenz. & G. Moreno D
Cortinarius macrorhizus Fr. D
Cortinarius multiformis (Fr.) Fr. P
Cortinarius napus Fr. P
Cortinarius obscurculoileus (M.M. Moser) Kühn.-Fink. & Peintner D
Cortinarius obtusus (Fr.) Fr. T
Cortinarius olivaceus Fr. P
Cortinarius olivaceofuscus Kühner D
Cortinarius orellanus Fr. L
Cortinarius parvannulatus Kühner T
Cortinarius perennis Fr. P
Cortinarius phaeopogmneus J. Favre T
Cortinarius phoeniceus (Vent.) Maire var. occidentalis A.H. Sm. D
Cortinarius phyllolepis (Wulf.) Fr. P
Cortinarius piceae Frasély, T.S. Jeppesen & Brandrud P
Cortinarius polidas Fr. L
Cortinarius porphyrophus (Alb. & Schwein.) Fr. P
Cortinarius pseudoglaucopus (Jul. Schaff. ex M.M. Moser) Quadr. D
Cortinarius raphanoides (Pers.) Fr. L
Cortinarius reinders Fr. T
Cortinarius rubellus Cooke L
Cortinarius rubicundulus (Rea) A. Pearson L
Cortinarius saginus (Fr.) Fr. P#
Cortinarius salor Fr. M
Cortinarius sanguineus (Wulfen) Fr. D
Cortinarius scareaus (Fr.) Fr. P
Cortinarius scoticus J. Favre T
Cortinarius semisanguineus (Fr.) Gillet D
Cortinarius stillatissus Fr. M
Cortinarius subalbinastrus Rob, Henry T
Cortinarius subtorquis (Pers.) Fr. P
Cortinarius sulphurinus Quél. P
Cortinarius tenuiceps M. Moller P
Cortinarius tailiae Brandrud P
Cortinarius tomentosus (Pers.) Fr. T
Cortinarius traganus (Fr.) Fr. S
Cortinarius triquhtamus Fr. P
Cortinarius turbinatus Fr. P
Cortinarius uliginosus (Fr.) D
Cortinarius vaciniohispinus Brandrud P
Cortinarius variicolor (Pers.) Fr. P
Cortinarius varius (Schaeff.) Britzelm. P
Cortinarius venetus (Fr.) Fr. L
Cortinarius vespertinus (Fr.) Fr. P
Cortinarius viridissilus Fr. M
Cortinarius violaceus (L.) Gray C#
numbers KC171232-KC171301 and KC842389-KC842529 (Table 1). In addition, sequences from 17 species were retrieved from the NCBI-nr nucleotide database (Table 1).

Alignments generated in this study have been made freely available through the authors ResearchGate pages (http://www.researchgate.net/).

Phylogenetic inference

The following species were pruned upon evaluation of the RogueNaRok-algorithm (Aberer et al. 2013): Cortinarius rubicundulus (Rea) A. Pearson, C. saginus, C. salor Fr., and C. callisteus (Fr.) Fr. However, pruning gave no additional support (data not shown). The inferred Cortinarius phylogenies (pruned or non-pruned analysed with ML or MrBayes, respectively) demonstrated good topological congruence with an $I_{long}$ P-value < 0.05. Removal of long-branching species, likewise, had minimal topological impact (data not shown). For this reasoning, the full dataset was preferred for visualization of the results. For interpretation of the phylogenetic inferences (Fig. 1), statistical support is defined as: full 1.00 posterior probability (PP)/100 bootstrap support (BS), high >90 BS, moderate >65 BS, and low >50 BS, highlighted with red lines in Fig. 1. Additionally, PP support over 0.95 in cases where BS ≤ 50 is highlighted with blue lines in Fig. 1.

The first and most basal clade to diverge from the main branch constituted C. austroauracinus M.M. Moser and C. renidens Fr. However, the basal position and the monophyly of these species were unsupported. The next grouping to diverge, again unsupported, was a lineage encompassing the terminal Scauri clade, recovered with moderate support (1.00/66). The following divergence was the Calochroi clade: The split was unsupported, however the grouping of the Calochroi clade was highly supported (1.00/96), as was the sister relationship between C. olearioides Rob. Henry and C. napus Fr. (1.00/94). The subsequent unsupported divergence was a large grouping harbouring the Phlegmacium and Myxacium lineages. The branching pattern between species was uncertain with lack of support for relationships apart from; the fully supported monophyly of the Myxacium clade, the low supported clade formed by C. glaukopis (Schaeff.) Fr., C. caerulescens (Schaeff.) Fr., C. terpsichores Melot, C. anserinus (Velen.) Rob. Henry, and C. viridicoerulesus Chevassut & Rob. Henry (0.98/52), and the moderate to highly supported sister relationships recovered between C. cumatilis Fr. and C. claricolor (Fr.) Fr. (1.00/96), C. olidus J.E. Lange and C. percomis Fr. (1.00/88), and C. balteatocumatilis Rob. Henry ex P.D. Orton and C. balteatus (Fr.) Fr. (1.00/98). The next species to split from the main branch was C. callisteus, subsequently followed by C. infractus, with both lacking support. The ensuing branch to split formed the unsupported Anomali clade. Only the

Figure 1. The rDNA phylogeny of Cortinarius.

ML tree for 86 Cortinarius species and four outgroup species (2373 rDNA characters). Red lines full (=100) to low BS (<50); blue lines PP ≥0.95 for BS ≤50. Main clades are named after subgenera or sections, sub-clades are named with letters.


Morphological, chemical, and ecological traits (blank = actual characteristic is lacking). Viscid: P = pileus, PS = pileus and stipe; spore shape: E = ellipsoid- amygdaloid, S = subglobose, C = citriniform; cuticle: S = simple, D = duplex, DA = duplex and amyloid, ? = no data; octaketide dimeric pre-anthraquinones: F = flavomannins, P = phlegnacins, H = hypericin and skyrin; octaketide monomeric anthraquinones: E = only endocrocin, EO = endocrocin and other anthraquinones, O = only other anthraquinones; nitrogen compounds: O = orellanine, C = endocrocin and other anthraquinones; monomeric anthraquinones: E = only endocrocin, EO = endocrocin and other anthraquinones, O = only other anthraquinones; nitrogen compounds: O = orellanine, C = endocrocin and other anthraquinones; monomeric anthraquinones; genome size: 7 = cylindrical cystidia, spores with plage, 8 = flesh purple in KI, 9 = crystallopicrin, 10 = basal mycelium staining rose, spores smooth, 11 = veil and cortina absent.

P = poor, M = medium, R = rich, C = calcareous, ? = no data; special characteristics: 1 = balloon shaped cells on pileus and stipe; spore shape: E = ellipsoid- amygdaloid, S = subglobose, C = citriniform; cuticle: S = simple, D = duplex, DA = duplex and amyloid, ? = no data; octaketide dimeric pre-anthraquinones: F = flavomannins, P = phlegnacins, H = hypericin and skyrin; octaketide monomeric anthraquinones: E = only endocrocin, EO = endocrocin and other anthraquinones, O = only other anthraquinones; nitrogen compounds: O = orellanine, C = endocrocin and other anthraquinones; monomeric anthraquinones; genome size: 7 = cylindrical cystidia, spores with plage, 8 = flesh purple in KI, 9 = crystallopicrin, 10 = basal mycelium staining rose, spores smooth, 11 = veil and cortina absent.
sister relationship of *C. anomalus* (Fr.) Fr. and *C. caninus* (Fr.) Fr. was confirmed with full support. The next grouping to diverge, again with lacking support, constituted four supported sister relationships; the fully supported *Orellani* clade, the fully supported *Leprocybe* clade, the moderately supported *Icterinula* clade (1.00/81), and the highly supported *Obtusi* clade (1.00/95). The subsequent unsupported divergence constituted a grouping where the fully supported *Pauperae* clade split first, recovering the highly supported *Dermocybe* clade (1.00/91). The ensuing divergence of *C. olivaceofuscus* Kühner from the main branch was inferred with low support (0.99/55). The subsequent split was highly supported (1.00/99), constituting the *Telamonia* clade. The branching pattern in the clade, however, was unclear with few sister relationships being recovered with support; *C. gentilis* with *C. brunnneus* (1.00/74), *C. pholideus* (Lilj.) Fr. with *C. raphanoides* (Pers.) Fr. (0.99/51), *C. phaeopyga* J. Favre with *C. laniger* Fr. (0.99/52), *C. traganus* (Fr.) Fr. with *C. subhalaustinus* Rob. Henry (0.99/57), *C. armeniacus* with *C. alboviclaveus* (1.00/96), and finally the fully supported *C. scotoides* J. Favre with *C. parvannulatus* Kühner.

**Discussion**

**Phylogeny**

Although the branching pattern between clades might be questionable due to uncertain phylogenetic signal, twelve infrageneric groups received satisfactory support (Fig. 1). These are: *Telamonia, Dermocybe, Pauperae, Obtusi, Icterinula, Leprocybe, Orellani, Anomali, Phlegmacium, Myxacium, Calochroï, and Scauri*. As mentioned in the introduction chapter most of these groups have recurrently been identified by several authors, e.g. (Høiland & Holst-Jensen 2000; Garnica et al. 2003a, 2003b, 2005; Peintner et al. 2004; Niskanen et al. 2008; Harrower et al. 2011). Our results confirm their findings, and except for *Anomali* and *Phlegmacium*, the phylogeny will not be discussed further.

Our analyses show that *C. olivaceofuscus* is recovered, with low BS, as a sister group to *Telamonia*, and we may discuss whether it should be included in *Telamonia* or not. Previously it has been included in *Dermocybe* section *Olivaceofusci* (Høiland 1984). With *Telamonia* it shares a hygrophanous pileus, with *Dermocybe* the octake-tide anthraquinones typical for this group (Høiland 1984, Gill & Steglich 1987). Based on morphology and pigment chemistry *C. olivaceofuscus* has only one known relative, *Dermocybe leptospermarum* E. Horak (not yet combined in *Cortinarius*) from New Zealand (Høiland 1984, Gill & Steglich 1987, Horak 1988, Keller et al. 1988). It is tempting to consider this small, strictly bi-Hemispheric group as an evolutionary link between *Dermocybe* and *Telamonia*, representing the ancestral state of the latter. The sequence of *C. olivaceofuscus* included in the *Dermocybe* clade by Garnica et al. (2005) is not matching this species, but is similar to *C. cinnamomeus* (BLASTn hit with 99 % identity and/or coverage, as of 09.2014).

The Bayesian inference supports a relationship between clade *Obtusi* and *Icterinula* (0.95/46), but no recognised morphological or chemical features combine these two clades. The same relationship was also shown by a previous analysis (Peintner et al. 2004).

*Clad* *Anomali* in its most narrow sense comprises *C. anomalus* and *C. caninus*, characterised by subglobose spores and dry basidiocarps often with glimmery pileus. *Anomali* was originally recognised as a section of subgenus *Telamonia* or alternatively subgenus *Sericeocybe* (Moser 1983, Niskanen et al. 2008). The European *C. spilomeus* (Fr.) Fr. and the South Hemispheric *C. tristis* E. Horak and *C. sclerophyllarum* Gasparini are added to this lineage (Garnica et al. 2005). According to the Bayesian tree, the clade can be broadened to include *C. salor* and *C. bolaris* (Pers.) Fr. (0.99/32). *Cortinarius salor* shows morphological similarity to *C. delibatus* Fr. by its slimy stipe and pileus, with which some earlier phylogenetic analyses indicate a possible relationship (Peintner et al. 2004, Harrower et al. 2011, Ryberg & Matheny 2011). Our result does not support this relationship. The inclusion of *C. bolaris* in clade *Anomali* seems, however, more justified based on morphological features (Garnica et al. 2005, Niskanen et al. 2008). A chemically unknown compound staining the flesh yellow, is characteristic for this species (Høiland 1980).

*Cortinarius infractus* is tentatively placed as a sister group to the assemblage spanning from clade *Telamonia* to *Anomali*. It has a *Phlegmacium*-like basidiocarp with a viscid pileus, subglo-
borne spores, and contains indole alkaloids (Brondz et al. 2007, Brondz & Høiland 2008), probably unique for *Cortinarius*.

It is worth noting that the Bayesian phylogeny gives support to a “superclade” (0.98/16) spanning from clade *Telamonia* to clade *Anomali*, including *C. infractus*. In this superclade we find the majority of species with soluble pigments of octaketide origin, all species with compounds of nonaketide origin, the majority of species with hygrophanous pileus, few species with viscid pileus, and no species with bulbous stipe base. Moreover, all species (except *C. salor*) have duplex pileus cuticle.

Interestingly, it is demonstrated that the ectomycorrhizal structure of species in clade *Dermocybe, Obtusi*, and *Leprocybe* possesses emanating hyphae closed by a clamp, a feature not seen in the other clades (although only a limited number of species were investigated) (Agerer 2006). This may confirm the relationship between these clades. Species from the other clades have emanating hyphae with open anastomoses, except for *C. caperatus* (Pers.) Fr. in which they are smooth with clamps (Agerer 2006).

Clade *Phlegmacium* is not supported by ML, however, the Bayesian inference provides support (0.97/28) for this large group spanning from the couple *C. balteatus* and *C. balteatocumatilis* to *C. glaucopus*. This clade may represent the retained part of subgenus *Phlegmacium* since *Cortinarius saginus*, the type species (Singer 1986), is a member of the group. Common for all these species is a viscid pileus, a dry stipe, and stout basidiocarps. Inside this clade ML gives support to some subordinate monophyletic groups marked a, b, c, and d in Fig. 1.

*Cortinarius balteatus* and *C. balteatocumatilis* (sub-clade a) are sister species with high BS support. Although not supported by ML or Mr. Bayes, both analyses are congruent in inferring a monophyletic relationship with *C. varicolor* (Pers.) Fr. and *C. vacciniophilus* Brandrud. This broadened concept of sub-clade a represents clade *Phlegmacioides* (Garnica et al. 2005).

*Cortinarius percomis* and *C. olidus* are monophyletic with moderate BS support (sub-clade b). This clade also includes species as *C. nanceiensis* Maire, *C. mussivus* (Fr.) Melot, and *C. papulosus* Fr. representing *Percomes* (Garnica et al. 2005). *Cortinarius percomis, C. nanceiensis,* and *C. mussivus* contain special octaketide pigments not found outside this group (Gill & Steglich 1987).

*Cortinarius claricolor* and *C. cumatilis* (sub-clade c) are sister species with high BS support, representing *Praestantes* (Garnica et al. 2005).

The group spanning from the couple *C. viridocoeruleus* and *C. anserinus* to *C. glaucopus* forms a monophyletic clade with low BS support (sub-clade d). A monophyletic relationship between these species in this, still unnamed, clade has also previously been demonstrated (Garnica et al. 2003b).

A number of species in our dataset are found in unsupported groups. *Cortinarius violaceus* contains a violet iron complex, unique for this species (von Nussbaum et al. 1998, Spitek et al. 2000). *Cortinarius caperatus* has, in addition to the membranous ring, an amyloid reaction in the cutis hyphae and the ectomycorrhizal mantle together with smooth emanating hyphae with clamps (Agerer 2006). Despite forming an unsupported monophyly, the very dissimilar morphological and chemical characteristics indicate that *C. violaceus* and *C. caperatus* are not closely related, thus this can be considered a topological artefact. The couple *C. subtortus* and *C. delibutus* constitute another unconfirmed monophyletic group. Common for both species is a slimy pileus and subglobose spores. *Cortinarius subtortus* has in addition prominent pleuro- and cheilocystidia and content of (iso)-quinoline alkaloids (Teichert et al. 2008). *Cortinarius delibutus* has additionally a slimy stipe. *Cortinarius rubicundulus* and *C. crassus* Fr. is by ML, but not by Mr. Bayes, put in an unsupported group. They share some morphological similarities such as stout basidiocarps and cystidia, but the spore ornamentation is different; indistinctly verrucose in *C. rubicundulus*, distinctly verrucose in *C. crassus*. Moreover *C. rubicundulus* contains a chemically unknown compound staining the flesh yellow, reminiscent to *C. bolaris* (Høiland 1980). *Cortinarius callisteus* is by ML put in an isolated, single position and by Mr. Bayes in an unsupported group together with *C. rubicundulus*. Unique for *C. callisteus* (and its close relative *C. citrinofulvescens* M.M. Moser) is a strong and peculiar smell of ozone (Niskanen et al. 2008). Both *C. callisteus* and *C. rubicundulus* have exceptionally long evolutionary branch lengths, as also shown by other authors (Peintner et al. 2004, Garnica et al. 2005, Harrower et al. 2011, Ryberg & Matheny 2011).

The species in clade *Scauri*, together with *C.
vespertinus (Fr.) Fr., C. viretus (Fr.) Fr., C. turgidus (Fr.) Fr., C. multiformis (Fr.) Fr., and C. limonius (Fr.) Fr., occupy basal positions in the phylogenetic tree. Few characteristics combine these species, but many of them have unique features not seen in the other species of the dataset (Fig. 1). For instance, the viscid pileus cuticle of C. viretus contains the intensely acrid triterpenoid crystallopicrin (Steglich et al. 1990). The most basal position is taken by the unsupported couple C. renidens (characterised by lacking veil and cortina) and the South American Nothofagus associated species C. austroduracinus. This monophyletic relationship is also indicated earlier (Garnica et al. 2005).

**Morphological characteristics**

A hygrophanous pileus is dominating among the species in clade Telamonia together with its sister C. olivaceofuscus. It is also met with in clades Obtusi and Myxacium, although weak in the last clade. It is worth noting that hygrophanity is also regular among the more basal species spanning from the couple C. austroduracinus and C. renidens to clade Scauri. Therefore we consider that hygrophanity is an ancestral character state in genus Cortinarius, which has been retained in some phylogenetic groups and reduced in other groups. The clades Dermocybe, Phlegmacium, and Calochroi are the most prominent examples of non-hygrophanous groups.

Conversely, a slimy pileus or a slimy pileus and stipe seem derived. This morphological feature seems to have developed independently on multiple occasions in our phylogeny and represents a key characteristic for clades Pauperae, Icterinula, Phlegmacium, Calochroi, and Scauri. The combination slimy pileus and stipe has developed four times in our phylogeny and is a key characteristic for clade Myxacium. It is tempting to believe that the slimy layers have developed to protect the basidiocarps against fungivorous insects and/or temporarily dry weather conditions.

A bulbous stipe base is also considered derived and has probably been evolved due to a pileocarp (subterranean) development as an adaptation to dry soils. It is found in all species in clade Calochroi and sub-clade d of clade Phlegmacium. In our phylogeny this characteristic has evolved on four separate occasions.

The ellipsoid to amygdaloid spores may represent the ancestral spore outline in genus Cortinarius since this outline is dominating in the lower branches of the tree (Fig. 1). Subglobose spores have evolved several times and do not seem to infer a good phylogenetic signal, except in clade Leprocybe and clade Anomali (in its widest sense). Citriniform spores are distinctive for clade Calochroi, but are also encountered in clade Phlegmacium (sub-clade d) and clade Myxacium. It could be speculated whether the relatively voluminous citriniform spores represent an adaptation to dry environments (Kauserd et al. 2011, Høiland 2012).

A duplex pileus cuticle may also represent an ancestral character state from which a simple cutis has evolved six independent times in our phylogeny. The simple cutis is distinctive for clade Calochroi and also for sub-clades a and b of clade Phlegmacium. Cortinarius caperatus is outstanding by its amyloid duplex cutis (Agerer 2006).

**Secondary chemistry**

The most conspicuous secondary compounds in genus Cortinarius are the anthraquinones of the octaketide biosynthetic pathway (Gill & Steglich 1987). They are distinctive for clades Dermocybe, Pauperae, Icterinula, and Leprocybe, but are also encountered in C. olivaceofuscus, and sporadically in clade Telamonia, Phlegmacium (sub-clade b), and Calochroi. Typical for clade Dermocybe (except C. sanguineus (Wulfen) Fr.), C. olivaceofuscus, and some species in clade Calochroi is content of dimeric pre-anthraquinones of flavomannin type. The dimeric pre-anthraquinones hypericin and skyrin are regularly found in clade Pauperae. In sub-clade b of clade Phlegmacium, dimeric pre-anthraquinones of phlegmacin type are found in C. percomis in our phylogeny, but they are also found in the related C. nanceiensis and C. mussivus (Gill & Steglich 1987, Garnica et al. 2005). Various monomeric anthraquinones, such as endocrocin, dermolutein, and demorubin, are found in all species of clade Dermocybe, Pauperae, Icterinula, and Leprocybe, and in C. olivaceofuscus (only endocrocin), and the two species of clade Calochroi that also contain flavomannin pre-anthraquinones. The monomeric anthraquinones emodin, dermoglaucin, and dermocycin are only found in clade Dermocybe. In clade Icterinu-
la and Leprocybe, together with C. cinnabarinus Fr. and C. armillatus (Fr.) Fr. in clade Telamonia, only monomeric anthraquinones are found, no dimeric pre-anthraquinones.

The anthraquinones may act as deterrents to insects and other animals feeding on basidiocarps since it has been demonstrated that anthraquinones in plants (many of them similar to those in fungi, e.g. emodin) have defensive effects upon insects or birds (Trial & Dimond 1979, Michael et al. 1997). Focusing on clade Dermocybe it is therefore interesting that the basal lines, spanning from C. malicorius to the couple C. semisanguineus (Fr.) Gillet and C. idahoensis Ammirati & A.H. Sm., have a broad range of anthraquinones, including emodin, demoglaucin, and dermocynbin. The aforementioned species are found in lowland boreal forests (Høiland 1984) rich in fungivorous insects, and they are lacking in arctic-alpine environments. Presumably, these anthraquinones may have been reduced in the derived lineage leading to the group spanning from C. croceus (Schaeff.) Gray to the couple C. uliginosus Berk. and C. polaris Høil. This group only contains endocrocin, dermolutein, and demorubin, which are universal for all members of Dermocybe, and penetrate the cooler, boreal to arctic-alpine environments with less fungivorous insects. For instance, the fungivorous Mycetophilidae, fungus gnats, are particularly species rich in lowland boreal North European forests, but with only few species in alpine environments (Søli & Kjerandsen 2008).

Compounds of nonaketide biosynthetic origin, i.e. the strongly fluorescent xanthones xanthenol, xanthenol, and the monomeric anthraquinone lepolutein, are distinctive for clade Leprocybe (Gill & Steglich 1987). However, these compounds (except lepolutein) are also found in one species, C. colymbadinus, in clade Telamonia. Like anthraquinones, the xanthones may act as feeding deterrents against insects, as demonstrated for plants (Larson et al. 2010).

An interesting question is whether the possession of the recorded octaketide and nonaketide compounds represents a derived or ancestral character state. Since the actual substances are not seen in the most basal lines in our phylogeny, we may hypothesise that the octaketide pigments were evolved after the split leading to species spanning from clade Telamonia to clade Calochroi, although the phylogenetic support for this split is limited. The nonaketide compounds may have been evolved later, perhaps after the split leading to species spanning from clade Telamonia to clade Orellani. Later, the biosyntheses of octaketides or nonaketides may have switched off in many evolutionary lineages, and sometimes replaced by biosyntheses of other compounds, such as substances with nitrogen. A hypothesis has been put forward that the possession of pigments in large quantities in species of Calochroi is an ancestral state for this clade, giving rise to species with less or no pigments (Froslev et al. 2007).

Clade Telamonia is, with few exceptions, remarkably “empty” concerning pigments with octaketide or nonaketide pathways. However, our phylogeny indicates that it probably has originated from fungi possessing such pathways, shown by its sister species, C. olivaceofuscus, and the few Telamonia species containing octaketide or nonaketide substances.

Secondary metabolites containing nitrogen are represented in clade Orellani, with the bipyridines orellane and orelline (Schumacher & Høiland 1983, Gill & Steglich 1987), in the single lines leading to C. infractus, with the indole alkaloids pre-infractin (β-carboline-1-propionic acid) and infractopierin (Brondz et al. 2007, Brondz & Høiland 2008), in C. subtortus with (iso)-quinoline alkaloids (Teichert et al. 2008), and in C. violaceus with an 1:2 iron (III) (R)-β-dopa complex (von Nussbaum et al. 1998, Spiteller et al. 2000). The metabolites infer good phylogenetic signals for the clades and species at hand, but their diverse chemical nature and erratic occurrence indicate that they have evolved several independent times via different biosynthetic pathways; probably as a response to environmental conditions. The bitter taste due to the alkaloids in C. infractus and C. subtortus may protect the basidiocarps from being eaten, since animals usually avoid this (Steglich et al. 1990, Spiteller 2008). The same applies to the non-nitrogen compound crystallopicrin in C. vibratilis. Orellane in C. rubellus and C. orellanus may have evolved as a response of enhanced soil acidity and liberation of aluminum ions in soil water since it is a good aluminum ion chelator (Høiland 1994).
Ecology

The majority of species in clade Telamonia and Dermocybe prefer poor, acidic coniferous or Betula forests or they are indifferent of forest type. The same applies to the few investigated species of clade Obtusi, Anomali, Myxacium, and Scauri. Several species of Telamonia and a few of Dermocybe, Obtusi, Anomali, and Myxacium have emerged from boreal forests into arctic-alpine environments where they established mycorrhiza with Salix, Betula nana, Dryas, or Bistorta vivipara (Høiland 1984, Gulden & Torkelsen 1996, Peintner 2008, Bjorbækmo et al. 2010, Geml et al. 2012). Clade Telamonia is remarkably species rich in boreal coniferous forests (Brandrud et al. 1990-2013; Niskanen et al. 2008). However, a common trait is that both are specialised to acidic soils (Brandrud et al. 1990-2013, Høiland 1994). Very few orellanine containing Cortinarius species are known on global scale, but they occur in both hemispheres (Moser & Horak 1975, Gasparini 2004). The split between C. orellanus and C. rubellus is set about 10 million years and the tentative stem for the Orellani group about 20 million years (Ryberg & Matheny 2011). Orellani may represent an isolated evolutionary lineage of which only a few species have survived up to now (Gasparini 2004).

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