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Telamonioid *Cortinarius* species of the *C. puellaris* group from calcareous *Tilia* forests

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Four species belonging to the *Cortinarius puellaris* group are presented, including the two new species *C. biriensis*, *C. subpuellaris* and the recently described *C. puellaris*. Based on type studies, it is shown that the fourth species in the group should be named *C. intempestivus* (=*C. cristatosporus*). The species co-occur and are all studied mainly from SE Norwegian calcareous *Tilia* forests, but at least some of them also occur in *Quercus* (-*Carpinus*) forests in temperate-mediterranean areas of C-S Europe, and are apparently widespread and much overlooked. These are all tiny, small, ochre-redbrown telamonioid cortinarii with strongly to spiny ornamented spores. Phylogenetically, the taxa are well-distinguished by 6 to 22 ITS differences. Together with two taxa only known from ectomycorrhizal ITS sequences, they constitute an apparently well-supported clade with uncertain affinity.

**Keywords:** Cortinariaceae, Agaricales, Basidiomycota, *C. puellaris* group, taxonomy, calcareous *Tilia* forests.

*Cortinarius* is a huge genus with a huge morphological variability. Especially among the small, hygrophanous species belonging to the morphologically circumscribed subgenus *Telamonia* (“telamonioid taxa”), there are still a lot of taxonomic complexes with a number of little collected, little understood and undescribed taxa, even in the most well-studied parts of Europe. An example of this is the here treated *C. puellaris* group, which has been almost completely unknown until quite recently. The *C. puellaris* group consists of (very) small and galerinoid, ochraceous brown to redbrown taxa, characterized by ellipsoid, strongly verrucose-spiny spores (cf. Brandrud et al. 2015).

Many *Cortinarius* species are confined to calcareous deciduous forests, and many occur together in often small hotspot sites of high conservation value. In SE Norway, calcareous, ancient *Tilia* (-*Corylus*) forests are the major hotspot habitat for this element, housing a number of regionally very rare *Cortinarius* species (cf. Brandrud et al. 2015). Our material is mainly from these small hotspot forests.

The data presented here is largely based on a monitoring program on calcareous *Tilia* forests of SE Norway as part of a national program for mapping and monitoring red listed species (the ARKO project), funded by The Norwegian Environment Agency. Some sequence data in this publication was generated in the Norwegian Barcode of Life Network (NorBOL) project, funded by the Research Council of Norway and the Norwegian Biodiversity Information Centre. Furthermore, some type sequences were generated as a part of a larger type study of *Telamonia* species (Liimatainen et al., unpubl).

**Materials and methods**

Morphological methods

Spores and other microscopic features were studied and measured with a 100 × oil immersion lens, with mounts in 2% KOH. From each basidiocarp, a random selection of ten to twenty mature spores obtained from cortina remnants/stipe surface was measured excluding apiculus and ornamentation. Mean values (MV) of spore length and width as well as Q-values (length/width ratio) were then calculated for each specimen. For type collections of new species, up to five different specimens were measured per collection.
Molecular methods

DNA was extracted from dried material (a piece of lamella) with the NucleoSpin Plant kit (Macherey-Nagel, Düren, Germany) or the Phire® Plant Direct PCR Kit (Thermo Scientific, USA) following the recommendations of the manufacturer.

The ITS region of the nrDNA was amplified with the primer pairs ITS1F/ITS4 or ITS1F/ITS4B (White et al. 1990, Gardes & Bruns 1993). Amplicons were sequenced at the University of Helsinki and at LGC Genomics (Berlin, Germany) with the same primers used in PCR reactions. The electropherograms were checked, assembled and edited with the CodonCodeAligner 4.1 (CodonCode Corporation, Centerville, MA, U.S.A).

Some sequences were generated in collaboration with the Norwegian Barcode of Life Network (NorBOL) as part of BOLD. For BOLD methods, see Ratnasingham & Hebert (2007, 2013).

Multiple sequence alignment was done by PRANK (Löytynoja & Goldman 2005) as implemented in its graphical interface (PRANKSTER) under default settings. After the manual adjustment in SeaView (Gouy et al. 2010) the phylogenetically informative indels were coded following the simple indel coding algorithm (Simmons et al. 2001) with the program FastGap 1.2 (Borchsenius 2009). Maximum Likelihood analysis was carried out using RAxML (Stamatakis 2014) in raxmlGUI (Silvestro & Michalak 2012). Rapid bootstrap analysis with 1000 replicates under the GTRGAMMA substitution model for DNA and default set for binary (indel) characters was used to test branch support.

Newly generated sequences have been deposited in GenBank and accession numbers are given in Tab. 1.

Results and discussion

The C. puellaris group is morphologically rather uniform. Based on the variation seen in the material studied so far, these are hardly possible to distinguish macroscopically. However, they are more differentiated in spore morphology features, and the two most frequent species (C. puellaris and C. intempestivus) can normally be distinguished on spore size when measured from spore deposits on stipe surface (see under Taxonomy). The taxa show a high similarity also phylogenetically, but all are well-distinguished with a clear barcoding gap, showing very little infraspecific variation and ≥ 8 differences in ITS sequences towards sister taxa. According to the available nrDNA ITS sequence data the group is still difficult to delimit at the moment.

Altogether six species are distinguished based on ITS sequence data (Fig. 1). Of these, two are so far known only from ectomycorrhizal samples, one from Quercus roots from Mexico, and the other from orchid roots in deciduous forests from Germany and France. These two species are not treated here, due to complete lack of morphological data from specimens, but they are included in the phylogenetic tree (Fig. 1).

Although the species are very poorly known, the group appears to be widely distributed in calcareous, thermophilous deciduous forests over most of Europe (S France–Italy to Norway). With an apparently overlapping morphological character variation towards at least one or two neighbours, but a well-supported ITS differentiation, the four species treated here should probably be regarded as semi-cryptic taxa. Two of them are described as new below. A phylogenetic tree is presented in Fig. 1 and relationships of sister species are further commented under each taxon.

Taxonomy

Cortinarius intempestivus Moënne-Loccoz & Reumaux, in Bidaud et al., Atlas des Cortinaires 11: 573. 2001. – Fig. 2
Mycobank no.: MB 374634

Pileus. – 0.5–2 cm, acutely conical–campanulate, later more umbrone, strongly hygrophanous and distinctly transluently striate; glabrous; from rather pale fulvous brown (“obtusus-colour”) to darker (red)brown to umbert brown; ochraceous white when dry; margin whitish from veil remnants when (very) young. – Lamellae crowded, adnate, edge even, initially pale ochraceous/fulvous brown, later darker (cinnamon) brown, edge often somewhat paler. – Stipe 3–7 × 0.2–0.4 cm, with (clavate–) cylindrical base; white fibrillose from sparse veil remnants when young; initially ochraceous white in upper part, soon ochraceous grey brown, and developing a dark clay brown colour from the base. – Context watery (grey) ochre brown when moist, darker brown in pileus, often darker brown in the lower half of the stipe; smell insignificant.

Basidiospores 9–10 × 5.5–6.5 µm, (MV=9.6 × 5.9 µm, Q = 1.63), (broadly) ellipsoid to somewhat drop-shaped in face view, very strongly and medi-
um coarsely verrucose, ornaments most prominent distally, often cristate-dentate. – Lamellae edge more or less sterile, with some small, clavate, hyaline sterile cells intermixed with basidia. Lamellae trama with distinct, yellow brown pigment; pigment mainly parietal, some narrow hyphae with zebra-striped encrustations. – Pileipellis duplex; epicutis thin, of 3–8 µm wide hyphae, with pale yellowish, membranal pigment, some hyphae basally also with zebra-striped, yellow-brown, encrusted pigment; subcutis (hypodermium) subcellular of inflated elements up to 25 µm wide, pigment yellow, parietal, amber-like, cementing the elements, encrusted in transition layers to epicutis.

**DNA (ITS) sequence.** Eight collections have been sequenced and all of them are identical.

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![Fig. 1. Phylogenetic tree inferred from nrDNA ITS sequences using RAxML. BS values > 50 % are indicated at branches. New species in bold face.](image)
Tab. 1. Details of sequences and specimens/strains included in the molecular and morphological analysis for the new species and interesting reports.

<table>
<thead>
<tr>
<th>Cortinarius species</th>
<th>Voucher numbers</th>
<th>Country</th>
<th>Locality</th>
<th>Substrate</th>
<th>Collector(s)</th>
<th>GenBank accession no. (ITS)</th>
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<td>C. biriensis</td>
<td>TEB 638-15, holotype</td>
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<td><em>Corylus avellana</em></td>
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except that in the sequence of TEB 235-14 one single indel difference was observed. *Cortinarius intempestivus* differs from *C. subpuellaris* and *C. biriensis* by 10 and 12 substitutions and indel positions, resp. *Cortinarius puellaris* is more dissimilar, with 20 substitutions and indel differences.

**Habitat.** – In Norway in boreonemoral, dry, calcareous *Tilia* forests, under *Tilia cordata* and *Corylus avellana* in leaf litter or more mineralic topsoil. In France in temperate *Quercus* forests as well as Mediterranean *Quercus ilex* sites.

**Distribution.** – Widely distributed along the Oslofjord, SE Norway. Also known from C France (Bidaud et al. 2001, 2004), and from S France (GenBank no. JF506769 as *Inocybe*).


**Comments.** – Based on the molecular studies of type specimens *C. cristatosporus* sensu Brandrud et al. (2015) is the same as *C. cristatosporus* Reumaux (Bidaud et al. 2004) and the latter is a younger synonym of *C. intempestivus* Möenne-Locc. & Reumaux (Bidaud et al. 2001). The protologue of *C. intempestivus* is not very representative for our taxon, and the spores are not described as spiny-cristate verrucose (Bidaud et al. 2001). The protologue of *C. cristatosporus* shows typical spores with more spiny ornaments at the distal end (at apex) (Bidaud et al. 2004).

*Corinarius intempestivus* is according to the material we have examined, the most cristate-spored species in the group, and most collections

<table>
<thead>
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<th>Collector(s)</th>
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Cortinarius puellaris Brandrud, Bendiksen & Dima, – Fig. 3
MycoBank: MB 814275

Description. – See Brandrud et al. (2015).


Comments. – Based on material studied so far (15 collections of C. puellaris and eight of C. intempestivus confirmed by sequencing), C. puellaris and C. intempestivus are macroscopically very similar, with a considerable intraspecific variability. However, the spores are more different, being smaller and more broadly ellipsoid in C. puellaris (average Q value = 1.49 versus 1.60 in C. intempestivus; Brandrud et al. 2015). All sequenced collections of C. puellaris and C. intempestivus collected in 2015 were correctly identified based on the spore morphology, indicating that it is normally possible to distinguish these two most frequent taxa in the group based on morphology.

Cortinarius biriensis Brandrud & Dima, sp. nov. – Fig. 4
MycoBank no.: MB 818220


Description. – Pileus 0.6–1.5 cm, conical-campanulate, later convex-umbonate, strongly hygrophanous and distinctly translucently striate; glabrous; fulvous, (red)brown to umber brown; pale ochraceous brown when dry. – Lamellae crowded, adnate, edge even, fulvous brown, later darker fulvous-cinnamon brown, edge somewhat paler. – Stipe 3–4.5 × 0.15–0.25 cm, with cylindrical, or with slightly tapering base; veil remnants very thin, seen as some diffuse, white fibers when young; initially ochraceous white in upper part, soon ochraceous (grey) brown in the lower half, and developing dark clay brown spots near the base. – Context watery (grey) brown when moist, darker brown in pileus, pale ochre when dry, some darker brown in the lower half of the stipe; smell insignificant.

Basidiospores (9)9.5–10.5 × (5.5)6–6.5 µm, (MV=9.89 × 6.19 µm, Q = 1.61), (broadly) ellipsoid to somewhat drop-shaped in face view, very strongly and medium coarsely verrucose, ornaments most prominent distally (but hardly cristate-dentate). – Lamellae edge more or less sterile, with some small, clavate, hyaline sterile cells intermixed with basidia. Lamellae trama with distinct, yellow brown pigment; pigment mainly paretal, some narrow hyphae eventually with zebra-striped encrustations. – Pileipellis duplex; epicutis thin, of 3–8 µm wide hyphae, with pale yellowish, membranous pigments, a few hyphae basally also sometimes with

Fig. 2. Cortinarius intempestivus, TEB 800-11, photo: K.H. Brandrud.

Fig. 3. Cortinarius puellaris, TEB 820b-11, photo: K.H. Brandrud.
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faint, zebra-striped, yellow, encrusted pigment; subcutis (hypodermium) subcellular of inflated elements up to 25 µm wide, pigment yellow(-brown), parietal, amber-like, cementing the elements. DNA (ITS) sequence. Three collections have been sequenced and they were identical. Cortinarius biriensis differs by 12 nucleotide and indel position from C. intempestivus, 13 from C. subpuellaris, and 22 from C. puellaris.

Habitat. – The species is found in one Norwegian locality (three different collections), in bore-onemoral, dry, calcareous Tilia forests, under Tilia cordata and probably some Corylus avellana in steep, scree area in leaf litter between stones. Furthermore, one Estonian locality with Corylus avellana on calcareous ground.

Distribution. – So far known from the type locality near lake Mjøsa, SE Norway and from one Corylus avellana ectomycorrhizal sample from Estonia (UBD025825).

Etymology. – The epithet refers to the type locality at Eriksrud nature reserve at Biri. This is a very rich outpost of Tilia forests, housing the worlds northermost occurrences of thermophilous cortinarii such as C. camptoros, C. insignibulbus and C. saporatus.


Comments. – The species has very small and tiny basidiomata. According to the three collections studied and ITS-verified so far, it is probably not possible to distinguish this species from C. intempestivus, C. puellaris and C. subpuellaris on macroscopical features. The specimens seen were not so prominently darkening from base as often seen in the other taxa, and the veil remnants were remarkably scanty, but these features might be variable. Microscopically, the species has spores rather similar to C. intempestivus (larger than those of C. puellaris), but the spores are less strongly verrucose-spiny and on average a little broader than those of C. intempestivus.

At the moment, C. biriensis is known only from a Tilia(Corylus) forest in Norway and a Corylus habitat in Estonia, but as with the related species C. intempestivus and C. puellaris, a wider habitat range including other kinds of calcareous deciduous forests can be expected.

Cortinarius subpuellaris Brandrud & Dima, sp. nov.

– Fig. 5
MycoBank no.: MB 818230


Description. – Pileus 0.5–1.5 cm, acutely conical to campanulate, later umbonate, strongly hygrophanous and distinctly translucently striate; glabrous to faintly silvery-fibrillose of veil remnants at centre when young; ochraceous brown-fulvous to darker (red)brown-umber brown; pale ochraceous brown when dry. – Lamellae crowded, adnate, edge even, initially rather pale ochraceous yellowbrown to fulvous brown, later rather pale brownish to darker (chocolate) brown, edge paler to sometimes whitish towards margin. – Stipe 2.5–4 × 0.2–0.3 cm, with (clavate-)cylindrical base, sometimes tapering; fibrillose of few, whitish veil remnants when young, rarely with a faint, white girdle; initially ochraceous white in upper
part, soon ochraceous grey brown, and developing a dark clay brown to blackish brown colour from the base and when bruised, contrasting the white basymycelium. – Context watery grey brown-ochraceous brown when moist, darker brown in pileus, soon becoming darker brown to spotwise blackish brown in the lower half of the stipe; smell insignificant.

Basidiospores 7.5–8.5 x 5–6 µm, (MV=8.07 × 5.62 µm, Q = 1.43), broadly ellipsoid to somewhat drop-shaped, very strongly and medium coarsely verrucose, ornaments most prominent distally, sometimes cristate-dentate. – Lamellae edge more or less sterile, with some small, clavate, hyaline sterile cells surrounded by longer basidia. Lamellae trama with distinct, yellow brown pigment; pigment mainly parietal, some narrow hyphae with zebra-striped encrustations; inflated elements up to 20–(25) µm wide. – Pileipellis duplex; epicutis thin, of 3–8 µm wide hyphae, with pale yellowish, membranal pigment, some hyphae basally also with distinct zebra-striped, yellow-brown, encrusted pigment; subcutis (hypodermium) subcellular of inflated elements up to 25 µm wide, pigment yellow-brown, parietal, amber-like, sometimes with more prominent crusts or “lenses” between the inflated elements, more narrow hyphae encrusted in transition layers to epicutis, a few narrow, oleiferous brown hyphae sometimes also seen intermixed.

DNA (ITS) sequence. Three collections have been sequenced and they were identical. Cortinarius subpuellaris differs by 10 nucleotide and indel position from C. intempestivus and 13 from C. puellaris as well as from C. bireniensis, but interestingly, the ITS1 region of C. subpuellaris is identical to the latter, thus all ITS differences appear only in the ITS2 region.

Habitat. – Boreonemoral, dry, calcareous Tilia-Corylus forests, under Tilia cordata and Corylus avellana on very shallow (mull) soil.

Distribution. – So far known from three localities along the Oslofjord, SE Norway; the type locality near Langesund, Telemark, outer Oslofjord, and localities near Slemmestad in the innermost Oslofjord and at Biri near Mjøsa.

Etymology. – The epithet refers to the strong morphological and genetic similarity to C. puellaris.


Comments. – Like the rest of the C. puellaris group, the species have small and tiny basidiomata. The species resembles C. intempestivus, C. puellaris and C. bireniensis with similar macroscopical features. The type specimens are turning more than average blackish brown from base, but this feature could vary with weather conditions, exposure, etc. Microscopically, however, the species is distinguished by its smaller, more broadly ellipsoid spores. More material is needed to see if the spore size and shape overlap slightly with the C. puellaris spores or not, but they are clearly smaller than those of C. intempestivus and C. bireniensis. The type of C. subpuellaris has also more strongly encrusted pileipellis hyphae than those seen in C. bireniensis and C. intempestivus.

The species might possibly be difficult to distinguish also from small specimens of taxa in the C. safranopes-hinnules groups, which often exhibit this kind of small, broadly ellipsoid, strongly verrucose spores, and occur in similar habitats. But these usually have an earth-like/dust-like smell, and/or more ochre yellow(-saffron) tinges on the stipe, and phylogenetically they seem to be more distant.

References

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