

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

Tor Erik Brandrud<sup>1,\*</sup>, Bálint Dima<sup>1,2,3</sup>, Kare Liimatainen<sup>2</sup>, Tuula Niskanen<sup>4</sup>

<sup>1</sup> Norwegian Institute for Nature Research (NINA), Gaustadalléen 21, NO-0349 Oslo, Norway

<sup>2</sup> Department of Biosciences, Plant Biology, P.O. Box 65, FI-00014, University of Helsinki, Finland

<sup>3</sup> Department of Plant Anatomy, Institute of Biology, Faculty of Science, Eötvös Loránd University, Pázmány Péter s. 1/c, H-1117 Budapest, Hungary

<sup>4</sup> Jodrell Laboratory, Royal Botanical Gardens, Kew, Richmond, Surrey, TW93DS, UK

\* e-mail: tor.brandrud@nina.no

Brandrud T. E., Dima B., Liimatainen K. & Niskanen T. (2017) Telamonioid *Cortinarius* of the *C. puellaris* group from calcareous *Tilia* forests. – *Sydowia* 69: 37–45.

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-reddish telamonioid cortinariid with strongly spiny ornamented spores. Phylogenetically, the taxa are well-distinguished by 8 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 map-

ping 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  $\geq 8$  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.

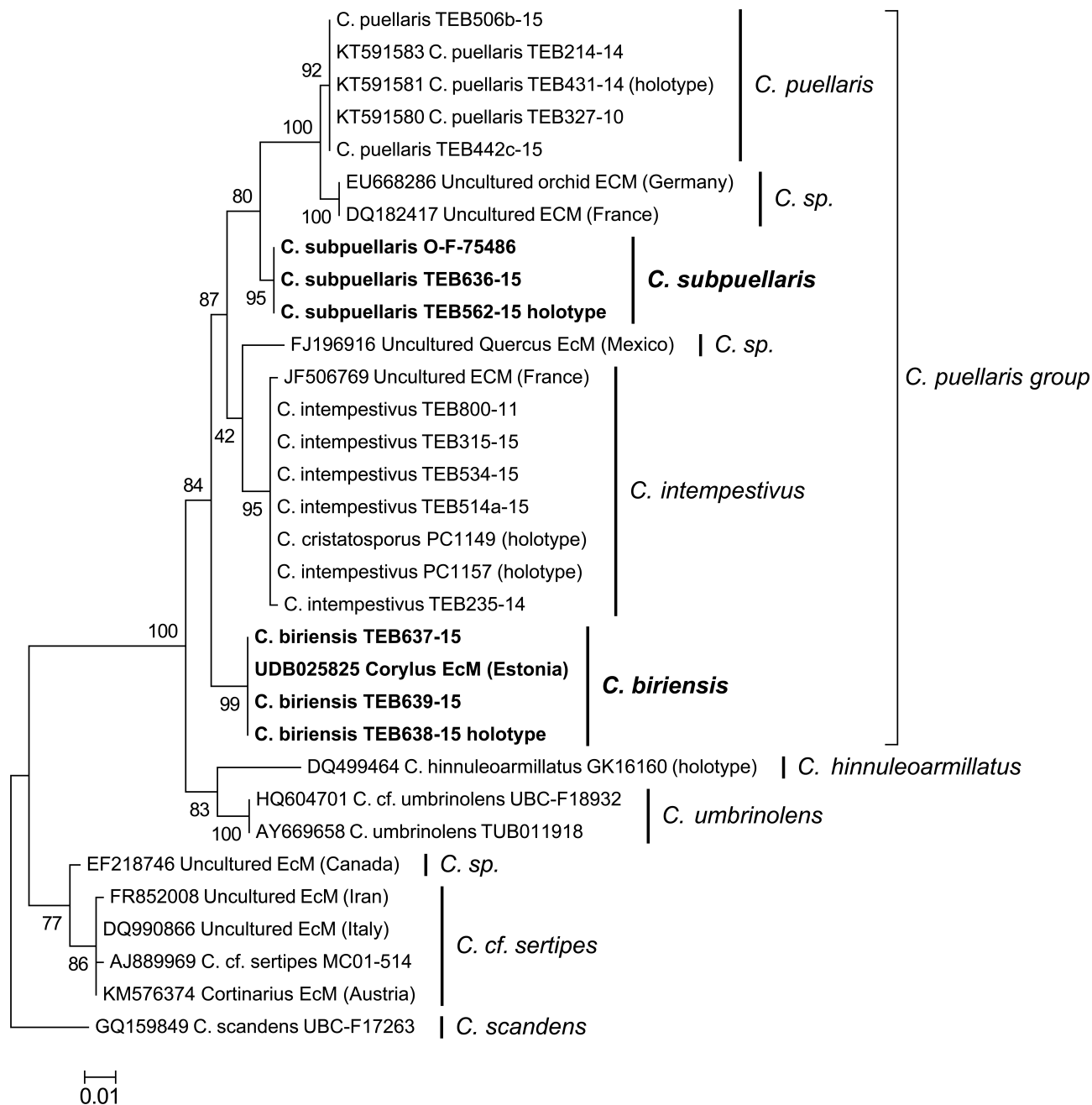
## Taxonomy

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

Synonym. – *Cortinarius cristatosporus* Reumaux, in Bidaud et al., *Atlas des Cortinaires* 14: 905. 2004. Mycobank: MB367631

**Pileus.** – 0.5–2 cm, acutely conical-campanulate, later more umbonate, strongly hygrophorous and distinctly translucently striate; glabrous; from rather pale fulvous brown (“obtusus-colour”) to darker (red)brown to umber 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-



**Fig. 1.** Phylogenetic tree inferred from nrDNA ITS sequences using RAxML. BS values > 50 % are indicated at branches. New species in bold face.

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  $\mu\text{m}$  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  $\mu\text{m}$  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

**Tab. 1.** Details of sequences and specimens/strains included in the molecular and morphological analysis for the new species and interesting reports.

<i>Cortinarius</i> species	Voucher numbers	Country	Locality	Substrate	Collector(s)	GenBank accession no. (ITS)
<i>C. biriensis</i>	TEB 638-15, holotype	Norway	Oppland county, Gjøvik, Biri	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima	KX831118
<i>C. biriensis</i>	TEB 637-15	Norway	Oppland county, Gjøvik, Biri	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima	KX831119
<i>C. biriensis</i>	TEB 639-15	Norway	Oppland county, Gjøvik, Biri	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima	KX886282
<i>C. biriensis</i>		Estonia	Saaremaa	<i>Corylus avellana</i>		UDB025825
<i>C. hinnuleoarmillatus</i>	GK16160, holotype	France		deciduous trees		DQ499464
<i>C. intemptivus</i>	PML 1157, holotype	France	Île de France, Versailles	deciduous forest	G. Martin	KX831120
<i>C. intemptivus</i>	PML 1149, holotype of <i>C. cristatosporus</i>	France	Loiret, Nogent-sur-Verneisson	<i>Quercus</i> forest	G. Redeuilh, P. Reumaux	KX831121
<i>C. intemptivus</i>	TEB 235-14	Norway	Oslo county, Oslo, Bygdøy	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima	KX831122
<i>C. intemptivus</i>	TEB 800-11	Norway	Buskerud county, Røyken	calcareous <i>Tilia</i> forest	T.E. Brandrud	KX831123
<i>C. intemptivus</i>	TEB 534-15	Norway	Buskerud county, Røyken	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima	KX831124
<i>C. intemptivus</i>	TEB 514a-15	Norway	Buskerud county, Røyken	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima	KX831125
<i>C. intemptivus</i>	TEB 315-15	Norway	Telemark county, Bamble	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima;	KX831126
<i>C. intemptivus</i>	LMAS2a-09, as Uncultured <i>Inocybe</i> clone	France	Puechabon state forest	<i>Quercus ilex</i>		JF506769
<i>C. puellaris</i>	TEB 431-14, holotype	Norway	Akershus county, Asker	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima;	KT591581
<i>C. puellaris</i>	TEB 506b-15	Norway	Akershus county, Asker	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima	KX831127
<i>C. puellaris</i>	TEB 442c-15	Norway	Akershus county, Asker	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima	KX831128
<i>C. puellaris</i>	TEB 214-14	Norway	Oslo county, Oslo, Bygdøy	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima	KT591583
<i>C. puellaris</i>	TEB 327-10	Norway	Telemark county, Bamble	calcareous <i>Tilia</i> forest	T.E. Brandrud	KT591580
<i>C. scandens</i>	UBC-F17263	Canada	British Columbia		O. Ceska	GQ159849
<i>C. cf.sertipes</i>	Ir15, as Uncultured <i>Cortinarius</i> clone	Iran				FR852008
<i>C. cf.sertipes</i>	as uncultured ectomycorrhiza ( <i>Cortinarius</i> )	Italy				DQ990866
<i>C. cf.sertipes</i>	MC01-514	Denmark	Sjaelland, Lille Boegeskov	<i>Fagus sylvatica</i>		AJ889969

<i>Cortinarius</i> species	Voucher numbers	Country	Locality	Substrate	Collector(s)	GenBank accession no. (ITS)
<i>C. cf. sertipes</i>	LM5406, as <i>Cortinarius</i> sp.	Austria		<i>Quercus petraea</i>		KM576374
<i>Cortinarius</i> sp.	M47D1, as Uncultured EcM ( <i>Cortinarius</i> ) clone	Mexico	Guerrero	<i>Quercus</i>		FJ196916
<i>Cortinarius</i> sp.	I.8238.3, as Uncultured <i>Cortinarius</i> isolate	Germany		orchid		EU668286
<i>Cortinarius</i> sp.	as uncultured Cortinariaceae isolate	France		<i>Cephalanthera longifolia</i>		DQ182417
<i>Cortinarius</i> sp.	UBCOCS392F, as Uncultured ectomycorrhiza ( <i>Cortinarius</i> ) isolate	Canada	British Columbia	<i>Pseudotsuga menziesii</i>		EF218746
<i>C. subpuellaris</i>	TEB 562-15, holotype	Norway	Telemark county, Bamble	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima	KX831129
<i>C. subpuellaris</i>	TEB 636-15	Norway	Oppland county, Gjøvik, Biri	calcareous <i>Tilia</i> forest	T.E. Brandrud, B. Dima	KX886283
<i>C. subpuellaris</i>	O-F75486	Norway	Buskerud, Røyken	calcareous <i>Tilia</i> forest	R. Braathen, E.W. Hanssen	KX831130
<i>C. umbrinolens</i>	TUB011918	Germany				AY669658
<i>C. umbrinolens</i>	UBC-F18932, as <i>C. cf. umbrinolens</i>	Canada	British Columbia		O. Ceska	HQ604701

except that in the sequence of TEB 235-14 one single indel difference was observed. *Cortinarius intemptivus* 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*).

**Material examined.** – FRANCE, Île-de-France, Parc de Versailles, under deciduous trees, Nov. 1988, leg. G. Martin, PML 1157 (Holotype of *C. intemptivus*, PC); Loiret, Nogent-sur-Vernisson, Arboretum des Barres, under *Quercus*, 12 October 1988, leg. G. Redeuilh & P. Reumaux, PML 1149 (holotype of *C. cristatosporus*, PC). – NORWAY, Oslo, Bygdøy, Hengsåsen, 29 August 2014, leg. T.E. Brandrud & B. Dima, TEB 235-14 (O). Akershus, Asker, Sjøstrandvegen, 23 September

2015, leg. T.E. Brandrud & B. Dima, TEB 506a-15 (O; partial sequence). Buskerud, Røyken, Bøsnipa, 13 September 2011, leg. E. Bendiksen, K. Bendiksen, T.E. Brandrud & I. Kytövuori, TEB 800-11 (O); Bøsnipa E, 24 September 2015, leg. T.E. Brandrud & B. Dima, TEB 534-15/DB5847 (O / BP). Slemmestadveien, 23 September 2015, leg. T.E. Brandrud & B. Dima, TEB 514a-15 (O). Telemark, Bamble, Høgenheitunnelen W, 9 September 2015, leg. T.E. Brandrud & B. Dima, TEB 315-15 (O).

**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. intemptivus* Möenne-Locc. & Reumaux (Bidaud et al. 2001). The protologue of *C. intemptivus* 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).

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



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

can be distinguished from *C. puellaris* by the size (and shape) of the spores (Brandrud et al. 2015). From the other large-spored, closely related species, *C. biriensis*, *C. intempestivus* can be separated by the more strongly verrucose-spiny spores, but there is considerable overlap in the spore morphology of these two species. Macroscopically *C. intempestivus* is very similar to the other members of the group (see comments on *C. puellaris*).

***Cortinarius puellaris*** Brandrud, Bendiksen & Dima, – Fig. 3  
Mycobank: MB 814275

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

**Material examined** (including ITS sequences; specimens studied after Brandrud et al. 2015): NORWAY, Akershus, Asker, Elnevangen SW, 18 September 2015, leg. T.E. Brandrud & B. Dima, TEB 410-15/DB5803, TEB 413-15 (O); Sjøstrandvegen, 23 September 2015, leg. T.E. Brandrud & B. Dima, TEB 506b-15 (O). Telemark, Porsgrunn, Åsstranda nature reserve, 28 September 2015, leg. T.E. Brandrud & B. Dima, TEB 594-15 (O); Blekebakken nature reserve, 25 September 2015, leg. T.E. Brandrud & B. Dima, TEB 555-15/DB5853 (O).

**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 infraspecific 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.



**Fig. 3.** *Cortinarius puellaris*, TEB 820b-11, photo: K.H. Brandrud.

***Cortinarius biriensis*** Brandrud & Dima, **sp. nov.** – Fig. 4

Mycobank no.: MB 818220

**Type.** – Norway, Oppland, Gjøvik, Biri, Eriksrud nature reserve, 30 September 2015, leg. T.E. Brandrud & B. Dima, TEB 638-15 (holotype, O). GenBank no. KX831118.

**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 parietal, some narrow hyphae eventually with zebra-striped encrustations. – Pileipellis duplex; epicutis thin, of 3–8 μm wide hyphae, with pale yellowish, membranous pigment, a few hyphae basally also sometimes with



Fig. 4. *Cortinarius biriensis*, sp. nov., holotype TEB 638-15, photo: B. Dima.



Fig. 5. *Cortinarius subpuellaris*, sp. nov., holotype TEB 562-15, photo: B. Dima.

faint, zebra-striped, yellow, encrusted pigment; subcutis (hypodermium) subcellular of inflated elements up to 25  $\mu\text{m}$  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. intemptivus*, 13 from *C. subpuellaris*, and 22 from *C. puellaris*.

**Habitat.** – The species is found in one Norwegian locality (three different collections), in boreonemoral, 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 (UDB025825).

**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 northernmost occurrences of thermophilous cortinariid such as *C. camptoros*, *C. insignibulbus* and *C. saporatus*.

**Material examined** (including ITS sequences). – NORWAY, Oppland, Gjøvik, Biri, Eriksrud nature reserve, leg. T.E. Brandrud & B. Dima, TEB 638-15 (holotype, O), TEB 637-15 (O), TEB 639-15 (O).

**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. intemptivus*, *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. intemptivus* (larger than those of *C. puellaris*), but the spores are less strongly verrucose-spiny and on average a little broader than those of *C. intemptivus*.

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. intemptivus* 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

**Type.** – NORWAY, Telemark, Bamble, Baneåsen nature reserve, 26 September 2015, leg. T.E. Brandrud & B. Dima, TEB 562-15 (holotype, O). GenBank No. KX831129.

**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  $\times$  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 basalmycelium. – 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 × 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, membranous 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. intemptivus* and 13 from *C. puellaris* as well as from *C. biriensis*, 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*.

**Material examined** (including ITS sequences). – NORWAY, Telemark, Bamble, Baneåsen nature reserve near Langesund, 26 September 2015, leg. T.E. Brandrud & B. Dima, TEB 562–15 (holotype, O); Buskerud, Røyken, Tåje near Slemmestad, 11 September 2014, leg. R. Braathen, E. W. Hanssen (O-F75486, O); Oppland, Gjøvik, Biri, Eriksrud nature reserve, leg. T.E. Brandrud & B. Dima, 30 September 2015, TEB 636–15 (O).

**Comments.** – Like the rest of the *C. puellaris* group, the species has small and tiny basidiomata. The species resembles *C. intemptivus*, *C. puellaris* and *C. biriensis* 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. intemptivus* and *C. biriensis*. The type of *C. subpuellaris* has also more strongly encrusted pileipellis hyphae than those seen in *C. biriensis* and *C. intemptivus*.

The species might possibly be difficult to distinguish also from small specimens of taxa in the *C. safranopes-hinnuleus* 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

- Bidaud A., Moëgne-Loccoz P., Reumaux P. (2001) *Atlas des Cortinaires*. Pars XI. S.A.R.L. Editions fédérations mycologique Dauphiné-Savoie. Marlioz.
- Bidaud A., Carteret X., Eyssartier G., Moëgne-Loccoz P., Reumaux P. (2004) *Atlas des Cortinaires*. Pars XIV. S.A.R.L. Editions fédérations mycologique Dauphiné-Savoie. Marlioz.
- Borchsenius F. (2009) *FastGap 1.2*. – [http://www.aubot.dk/FastGap\\_home.htm](http://www.aubot.dk/FastGap_home.htm), Department of Bio-sciences, Aarhus University, Denmark.
- Brandrud T.E., Bendiksen E., Dima B. (2015) Some new and little known telamonioid *Cortinarius* species from Norway. *Agarica* **36**: 11–42.
- Gardes M., Bruns T.D. (1993) ITS primers with enhanced specificity for basidiomycetes, application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.
- Gouy M., Guindon S., Gascuel O. (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology & Evolution* **27**(2): 221–224.
- Löytynoja A., Goldman N. (2005) An algorithm for progressive multiple alignment of sequences with insertions. – *Proceedings of the National Academy of Sciences of the U.S.A.* **102**(30): 10557–10562.
- Ratnasingham S., Hebert P.D.N. (2007) BOLD: The Barcode of Life Data system ([www.barcodinglife.org](http://www.barcodinglife.org)). *Molecular Ecology Notes* **7**, 355–364. DOI: 10.1111/j.1471-8286.2006.01678.x
- Ratnasingham S., Hebert P.D.N. (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN)



- System. *PLoS ONE* **8**(8): e66213. DOI:10.1371/journal.pone.0066213
- Silvestro D., Michalak I. (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* **12**: 335–337. DOI 10.1007/s13127-011-0056-0
- Simmons M.P., Ochoterena H., Carr T.G. (2001) Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analysis. *Systematic Biology* **50**(3): 454–462. doi: 10.1080/106351501300318049
- Stamatakis A., (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313. doi: 10.1093/bioinformatics/btu033
- White T.J., Bruns T., Lee S., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J. (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322.
- (Manuscript accepted 7 September 2016; Corresponding Editor: I. Krisai-Greilhuber)

