

Cortinarius gueneri: A new species from the Euro-Siberian Floristic Region of Turkey

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Abstract

Cortinarius gueneri (subgen. *Telamonia* sect. *Laeti*) is described as a new species from Turkey based on morphological and molecular data. It can be recognized by the somewhat irregular conical to campanulate, translucent-striate, rusty brown pileus; pale yellowish to cinnamon brown broadly attached lamellae; pale salmon to pinkish brown stipe; narrowly amygdaloid to ellipsoid basidiospores; and its putative association with *Carpinus orientalis*. Full description of the new species is given with field photos, microscopic illustrations, and a short discussion. Phylogenetic analysis of the internal transcribed spacer (ITS) region is also provided.

Introduction

Cortinarius (Pers.) Gray is the largest genus of mushroom-forming fungi, including more than 3000 species. The generic name originates from the distinctive cortinas that cover their lamellae when young, and rusty-brown spores further distinguish the genus. Members of *Cortinarius* have a cosmopolitan distribution, are terrestrial, and are important ectomycorrhizal mushrooms associated with both conifers and deciduous trees. Traditionally, the genus has been divided into subgenera based on the pileus and stipe surfaces, the shape of the stem, and whether basidiomata are hygrophane. However, some molecular phylogenetic studies of the genus do not fully support these morphological classifications (Peintner et al. 2004, Liimatainen et al. 2014). The phylogenetic analysis by Peintner et al. (2004) revealed seven well supported clades including *Myxacium* s.l., subg. *Cortinarius*, the phlegmacioid clade, the calochroid clade, the telamonioid clade, *Dermocybe* s.l. and *Myxotelamonia*. According to Liimatainen et al. (2014), *Cortinarius* is a morphologically and taxonomically challenging genus, in which some species names have not been used consistently and some species have been described two or more times under separate names.

The Euro-Siberian Floristic Area includes the Eastern Black Sea Region of Turkey and is covered with forests consisting of various deciduous and evergreen trees, including *Abies bornmuelleriana* Matf., *Alnus glutinosa* (L.) Gaertn., *Carpinus betulus* L., *C. orientalis* Mill., *Corylus avellana* L., *Fagus orientalis* Lipsky, *Picea orientalis* (L.) Peterm., *Pinus sylvestris* L., *Quercus petraea* (Matt.) Liebl., *Rhododendron luteum* Sweet and *Rh. ponticum* L. (Sesli & Denchev 2014). In addition to the rich plant diversity, a wide range of fungal species grow in the region. According to a recent compilation study resulting in a species checklist, 5,865 fungal species have been reported in Turkey, of which 130 are *Cortinarius* (Sesli et al. 2020). Within the same project, a unique Turkish name was given to each taxon according to expert input (Güner et al. 2020).

Despite the genus' reputation for taxonomic difficulty, some global-scale studies of the genus have been reported recently (Soop et al. 2019, Liimatainen et al. 2020). We previously collected and

described another species of *Cortinarius* from the region, *C. conicoumbonatus* E. Sesli, Liimat. & K. Demirel (Sesli & Liimatainen 2018). The purpose of the present study is to introduce a new species of *Cortinarius* to science.

Materials & Methods

Sampling and morphological studies

Basidiomata were collected from Mataracı, Trabzon, Turkey. Photographs were taken in situ. The plant diversity at the collection site was noted; the size, colour and the external morphological structure of the pileus, stem and the lamellae were recorded. One of the basidiomata was used to obtain spore prints and the others were dried for future microscopic study. Thin, freehand, superficial cross sections were obtained from the pileus and lamellae under binocular microscope (Niskanen & Kytövuori 2008), treated in ammonia solution, stained with Congo red, and photographed under a Zeiss Axio Imager A2 trinocular microscope. Basidiospores, basidia, marginal cells, and pileipellis were examined with Zeiss Axiovision version 4.8. The description was derived from Moser et al. (1995), Bidaud et al. (1997), Høiland and Holst-Jensen (2000), Moser (2002), Bidaud et al. (2006) and Niskanen & Kytövuori (2008). Type materials were deposited in the Karadeniz Technical University Forestry Faculty Herbarium (KATO 21965), Trabzon, Turkey, and the alignment employed for the phylogenetic analysis is available as supplementary material.

DNA extraction, PCR amplification, and sequencing

Total DNA was extracted from dried specimens employing a modified protocol based on Murray & Thompson (1980) by ECOTECH Biotechnology, Research and Development Company, Erzurum, Turkey. Primers ITS1F and ITS4 (White et al. 1990; Gardes & Bruns 1993) were used to amplify the ITS rDNA region. The polymerase chain reaction (PCR; Mullis & Faloona 1987) included 35 cycles with an

Table 1. Taxon names and GenBank numbers used in the phylogenetic analyses

Accession numbers	Cortinarius taxa	References
EU821676	<i>C. 'azureus' Fr.</i>	Harrower et al. (2011)
NR131815	<i>C. badiovinaceus</i> M.M. Moser	Niskanen et al. (2012)
JQ746617	<i>C. bulliardioides</i> Rob. Henry	Niskanen et al. (2012)
KX388647	<i>C. bulliardioides</i> Rob. Henry	Niskanen et al. (2012)
AY033096	<i>C. collinitus</i> (Sowerby) Gray	Peintner et al. (2002)
MW346633	<i>C. duracinobtusus</i> Rob. Henry	This work
FJ717565	<i>C. fillionii</i> Bidaud, Moëgne-Loec. & Reumaux	Harrower et al. (2011)
JQ746616	<i>C. floccopus</i> Bidaud	Niskanen et al. (2012)
NR121537	<i>C. floccopus</i> Bidaud	Schoch et al. (2014)
KX388637	<i>C. fulvescens</i> Fr.	Niskanen et al. (2012)
NR153077	<i>C. fulvescens</i> Fr.	Niskanen et al. (Unp.)
UDB018657	<i>C. fulvescens</i> Fr.	(Unpublished)
MW346632	<i>C. gueneri</i> sp. nov.	This work
FJ039604	<i>C. ochrophyllus</i> Fr.	Harrower et al. (2011)
FJ039605	<i>C. ochrophyllus</i> Fr.	Harrower et al. (2011)
KX388641	<i>C. pseudobulliardioides</i> Kytöv., Niskanen, Liimat. & Ammirati	Niskanen et al. (2012)
KX388642	<i>C. pseudobulliardioides</i> Kytöv., Niskanen, Liimat. & Ammirati	Niskanen et al. (2012)
AB848455	<i>Cortinarius</i> sp.	Miyamoto et al. (2014)
LC373242	<i>Cortinarius</i> sp.	Vaario et al. (2019)
GQ159906	<i>C. subfloccopus</i> Kytöv., Niskanen & Liimat.	Harrower et al. (2011)
JQ746609	<i>C. subfloccopus</i> Kytöv., Niskanen & Liimat.	Niskanen et al. (2012)
JQ746615	<i>C. subfloccopus</i> Kytöv., Niskanen & Liimat.	Niskanen et al. (2012)
NR120098	<i>C. subfloccopus</i> Kytöv., Niskanen & Liimat.	Schoch et al. (2014)
GQ159858	<i>C. tenuifulvescens</i> Kytöv., Niskanen & Liimat.	Harrower et al. (2011)
KX388644	<i>C. tenuifulvescens</i> Kytöv., Niskanen & Liimat.	Niskanen et al. (2012)
AB251817	ectomycorrhiza	Lian et al. (2006)
JQ393043	ectomycorrhiza	Kennedy et al. (2012)
LC175526	ectomycorrhiza	Miyamoto et al. (2018)
UDB032440	soil	(Unpublished)
UDB0336811	soil	(Unpublished)

annealing temperature of 54 °C. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked for putative base calling errors, and these were manually corrected.

Sequence alignment and phylogenetic analysis

BLAST (Altschul et al. 1990) was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration (INSDC, Cochrane et al. 2011) and UNITE (Nilsson et al. 2018) public databases. The sequences employed (Table 1) were aligned in MEGA 5.0 (Tamura et al. 2011) with Clustal W and adjusted manually. The final alignment, which included 112/568 variable/total sites among 30 sequences, was loaded in MrBayes 3.2.6 (Ronquist et al. 2012), where a Bayesian analysis was performed (data not partitioned, two simultaneous runs, four chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after 0.86 M generations, standard deviation having fell below 0.01. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML 8.2.12 (Stamatakis 2014) using the standard search algorithm (data partitioned, GTRGAMMA model, 2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Results

Phylogenetic analysis

The closest matches in public databases to the ITS sequences obtained from the specimen analysed came from collections of *Cortinarius* sect. *Laeti* Melot (clade *Fulvescentes* Melot in Niskanen et al. 2012), with a maximum similarity of 98.90% with *Cortinarius* sp. Fr. (EU821676). A phylogenetic reconstruction of this section resulted in a significant support for a monophyletic origin of the studied samples with *C. 'azureus'*, *C. duracinobtusus* Rob.

Henry, as well as several undetermined collections from public databases. Due to its morphological and ecological traits, which are distinct from those of *C. duracinobtusus* and other species described so far that lack genetic data, a new name is proposed below to accommodate it. GenBank codes are ALV21950 = MW346632 for *Cortinarius gueneri* (Fig. 1) and ALV21958 = MW346633 for *C. duracinobtusus*.

Taxonomy

Cortinarius gueneri E. Sesli, sp. nov. –Fig. 2
IF no: IF558131

DIAGNOSIS: Distinguished by somewhat irregular, more or less conical to campanulate, umbonate, hygrophanous, translucent-striate, rusty brown or ochre-brown to dark orange-brown pileus; pale yellowish to cinnamon brown and broadly attached lamellae; pale salmon to pinkish brown and fibrillose stipe; narrowly amygdaloid to ellipsoid, slightly verrucose basidiospores.

TYPUS: TURKEY. Trabzon, Maçka, Mataracı, N 40°50'51.22", E 39°37'39.88", 700 m alt., in mixed deciduous forest on soil, 27 Oct. 2016, E. Sesli, ALV21958 - KATO 21965, Sesli 3822. GenBank Acc. No. MW346632.

ETYMOLOGY: This name was given in honour of Prof. Dr. Adil Güner who devoted almost his entire life to the study of Turkish biodiversity at various universities and research institutions, including the Nezahat Gökyiğit Botanical Garden (NGBB) of Ali Nihat Gökyiğit Foundation (ANG Vakfı).

PILEUS 30–40 mm, somewhat irregular, at first more or less conical to campanulate and obtusely umbonate, later tending to be flatter with an obtuse umbo, in places concave or crested; margin undulate. Surface hygrophanous, translucent-striate up to halfway to the center; rusty brown, deep henna or ochre-brown when moist, ochre-brown to dark orange-brown when dry. Context very thin (almost

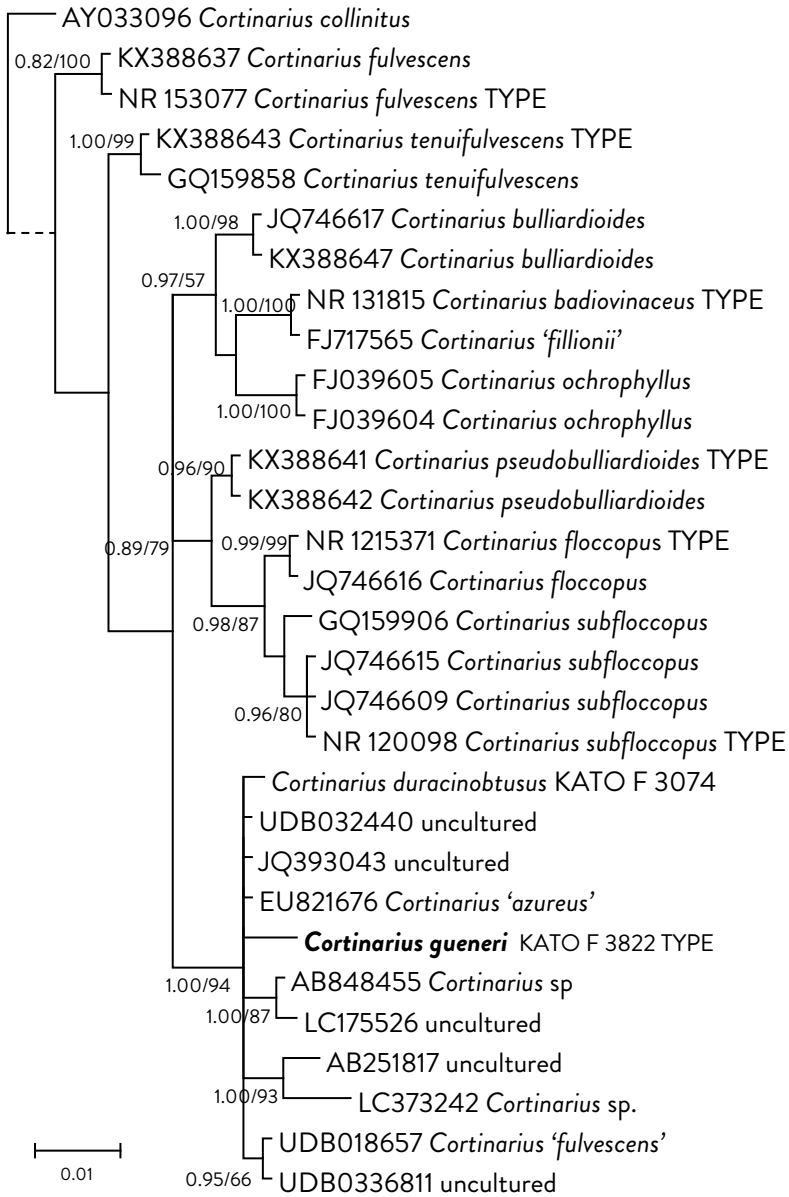


Fig. 1. 50% majority rule ITS rDNA consensus phylogram of *Cortinarius* section *Laeti* (with *C. collinitus* as outgroup) obtained in MrBayes from 2700 sampled trees. Nodes were annotated if supported by ≥ 0.95 Bayesian PP (left) or $\geq 70\%$ ML BS (right).

as thin as a lamella except where it is attached to the pileus), brown when moist, creamy to yellowish brown when dry; smell indistinct. Lamellae pale mandarin, pale yellowish to cinnamon brown; almost thick, medium to wide spaced; notched and broadly attached; L= 27–37, I=1–4, edge entire to rarely crenate. STIPE 30–50 × 5–9 mm, cylindrical to clavate, sometimes eccentric; slightly larger at the base and at the top; almost solid; light or pale salmon, cream to pinkish brown; longitudinally fibrillose; covered with a typical whitish mycelium near base.

BASIDIOSPORES [n= 100] (6.7–)7.5–8.5(–10.2) × (4.3–)5–5.5(–6.9) μm, on average 8 × 5.2 μm, light ochre, olive or greenish-yellow; narrowly amygdaloid to ellipsoid, slightly verrucose, Q= 1.4–1.6. Basidia cylindrical to clavate, 25–35(–40) × 7.3–9.5(–10.3) μm [n= 30], with 4(2) sterigmata and a clamp connection, some with granular contents. **MARGINAL CELLS** cylindrical to clavate and similar to basidiole, (15.1–)20–27(–31.5) × (6.4–)8–10.1 μm [n= 30]. **PILEIPELLIS** composed of hyaline to light brown, in part slightly encrusted, 7–11(–12.5) μm periclinal hyphae; clamp connections present; elements of subpellis 80–190 × 30–60 μm.

ADDITIONAL MATERIALS EXAMINED: Turkey. Trabzon, Maçka, Mataracı in mixed deciduous forest, 4 Nov. 2019, KATO 21965b, E. Sesli, Sesli 4309. Habit, habitat, and distribution – Gregarious in mixed deciduous forest. Putatively mycorrhizal with eastern hornbeam (*Carpinus orientalis* Mill.). Fruiting in autumn. Known only from East Black Sea Region of Turkey so far.

Discussion

Cortinarius gueneri belongs to *Cortinarius* subgen. *Telamonia* sect. *Laeti*. According to Soop et al. (2019) and Garrido-Benavent et al. (2020) sect. *Laeti* and sect. *Fulvescentes* are synonyms, and sect. *Laeti* has priority. *Cortinarius gueneri* differs from closely related species (Table 2) by the irregular, conical to

campanulate and obtusely umbonate, 30–40 mm wide, striate, rusty-brown, deep henna or ochre-brown to dark orange-brown pileus; by the very thin, brown to cream or yellowish brown context; the pale mandarin to cinnamon brown, notched, entire lamellae; the cylindrical to clavate or sometimes eccentric, light or pale salmon, cream to pinkish brown, 30–50 × 5–9 mm stipe; 7.5–8.5 × 5–5.5 μm, narrowly amygdaloid to ellipsoid, slightly verrucose basidiospores; 25–35 × 7.3–9.5 μm basidia and 20–27 × 8–10 μm marginal cells. *Cortinarius fulvescens* differs with longer basidiospores (7.5–9.5 μm), slightly larger (25–50 mm) and red brown to vinaceous pileus, longer stipe (50–120 mm), smaller basidia (23–28 × 6.5–8 μm) and narrow marginal cells (15–30 × 5–7.5 μm) (Niskanen & Kytövuori 2008). *Cortinarius ochrophyllus* has larger basidiomata (35–65 mm pileus and 50–130 × 5–11 mm stipe), different pileus color (pale greyish to yellowish brown) and different basidiospores (subglobose to broadly ovoid) (Niskanen & Kytövuori 2008). *Cortinarius bulliardoides* has typically larger (8.5–10.5 × 6–7 μm) and broadly ovoid basidiospores, longer stipe (60–120 mm) and red brown to vinaceous red brown pileus (Niskanen & Kytövuori 2008). Another phylogenetically close species, *C. badiovinaceus* differs by vinaceous red brown pileus, slightly shorter (5.5–7.5 μm) and ovoid-subglobose basidiospores (Niskanen & Kytövuori 2008, Soop 2018). *Cortinarius subfloccopus* has red brown to vinaceous red brown and slightly smaller (15–40 mm) pileus, longer (50–110 mm) stipe and typically larger (9–10.5 × 5.5–6.5 μm) basidiospores (Niskanen & Kytövuori 2008). Another newly recorded and described species from Turkey which was sequenced in this study, *C. duracinobtusus*, has a conical to campanulate, umbonate, smaller, brown pileus; sub-decurrent to adnate, sparse, dark yellow to orange brown lamellae; slightly larger stipe; ellipsoid and slightly verrucose basidiospores (Sesli & Örtücü 2020). Another similar species, *C. fulvescentoides*, differs with smaller (10–25 mm) warm red-brown pileus and longer (40–100 mm) stipe (Hyde et al. 2016). *Cortinarius tenuifulvescens* has a smaller (10–30 mm) warm red-brown pileus, longer stipe (50–110 mm), narrowly ellipsoid basidiospores and dark red-brown context (Hyde et al. 2016). *Cortinarius nymphatus* Kytöv., Niskanen, Liimat. & Bojantchev differs by smaller (10–30 mm) matte brown to dark

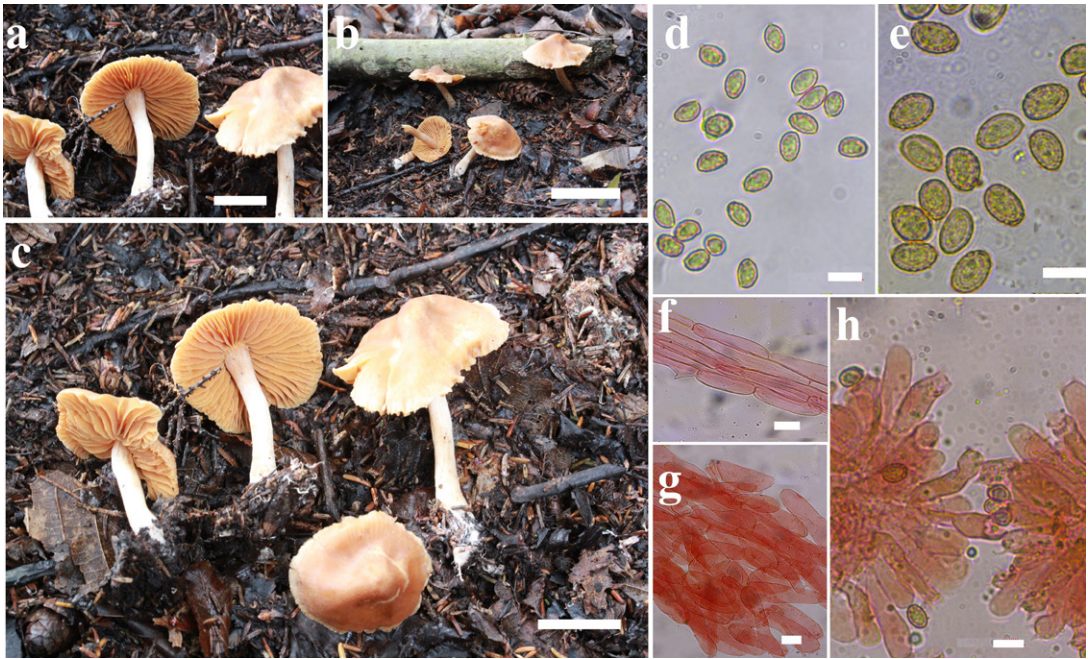


Fig. 2. *Cortinarius gueneri*. **a, b, c:** basidiomata. **d, e:** basidiospores. **f:** elements of stiptipellis. **g:** elements of pileipellis. **h:** basidia and basidioles (all from holotype). Scale bars: a, c = 20 mm, b = 40 mm. d, e, h = 10 μm . f, g = 20 μm .

red-brown pileus and smaller basidiospores (6.8–8.2 \times 4.3–4.8 μm) (Hyde et al. 2016). *Cortinarius pseudobulliardoides* has a matte, dark reddish-brown pileus and larger (8.4–10 \times 5.5–6.6 μm) basidiospores (Hyde et al. 2016).

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