



## Three new records of *Cortinarius* (Cortinariaceae) for the Turkish Mycota from Trabzon, Turkey

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### Abstract

Basidiomata of *Cortinarius* were collected during the field studies in 2019 and identified based on both morphological and molecular data. Three *Cortinarius* species viz. *Cortinarius cadi-aguirrei*, *C. cinnamoviolaceus* and *C. variegatus* are recorded for the first time from Turkey. Full descriptions, color figures, a simple phylogenetic tree and a short discussion are provided here.

**Key words** – Agaricales – Basidiomycota – ITS – identification – taxonomy

### Introduction

Interesting *Cortinarius* species have been described worldwide at recent years. Some new and little known telamonioid *Cortinarius* species described from Norway by Brandrud et al. (2016). A new conifer-associated, darkening species in the subgenus *Telamonia*, *Cortinarius badiolaevis* Niskanen, Liimat., Mahiques, Ballarà, Kytöv. was described from Sweden, Upland, Närke commune, Lillkyrka parish, Ekebergsbrottet in coniferous forest on calcareous soils (Niskanen et al. 2011b). Two new species of *Cortinarius*, subgenus *Telamonia*, sections *Colymbadini* and *Uracei*, from Europe described by Dima et al. (2014). The largest type study of Agaricales species to date was conducted by Liimatainen et al. (2014). Another important study related with our study is *Cortinarius* sect. *Armillati* in northern Europe by Niskanen et al. (2011a). Before the present study, *Cortinarius cadi-aguirrei* Garrido-Ben., Ballarà & Mahiques, *Cortinarius cinnamoviolaceus* M. M. Moser and *Cortinarius variegatus* Bres. were known from Spain, France and Finland respectively.

*Cortinarius cadi-aguirrei* Garrido-Ben., Ballarà & Mahiques was collected and described from Spain, Lleida, Isil and Alòs, Bordesd'Alòs in 2015. It is characterised with slender, relatively small, orange, reddish orange to apricot-like pileus; pale creamy, small stipe and mostly ellipsoid, sometimes slightly amygdaliform basidiospores (Garrido-Benavent et al. 2015). *Cortinarius cinnamoviolaceus* M. M. Moser was described by Bellanger et al. (2017) from France under *Picea abies* Karsten in 2012 and is easily distinguished by purple-brown pileus and narrow amygdaliform to ellipsoidal basidiospores. *Cortinarius variegatus* Bres. was described by Giacopo Bresàdola (Bresadola 1884). In our study, we found *Cortinarius cadi-aguirrei*, *C. cinnamoviolaceus* and *C. variegatus* from Turkey for the first time and are described as new records from Turkey.

## Materials & Methods

### Collecting site and morphological description

The East Black Sea region of Turkey is rainy during whole year, precipitation reaches its maximum in spring and autumn. The annual precipitation shows a significant increase from the west towards the east. On the north coast of Turkey, the differences between summer and winter temperatures are not too much. About 30% of the region was originally covered by forests. *Fagus orientalis* Lipsky and *Quercus petraea* (Mattuschka) Liebl. forests in the west are replaced by *Pinus sylvestris* L. and *Abies bornmuelleriana* Mattf. in the East. *Picea orientalis* (L.) Link becomes dominant above 1000 m. *Picea orientalis* (L.) Link, *Carpinus betulus* L., *C. orientalis* Mill., *Castanea sativa* Mill., *Rhododendron ponticum* L., *Rh. luteum* Sweet, *Corylus avellana* L. and *Alnus glutinosa* (L.) Gaertn. are the most common species in the eastern part of the Black Sea coastlands. The alpine biome within the East Black Sea region extends above 2000 m. The Kaçkar Mountains in the east support alpine elements from the Anatolian and Caucasian alpine regions as well as many endemic taxa confined to the East Black Sea region. Dried specimens are deposited in a personal herbarium of Trabzon University, Trabzon, Turkey. Facesoffungi numbers were registered as in Jayasiri et al. (2015).

Basidiomata were collected and photographed from Akçaabat and Maçka districts of Trabzon province of Turkey. Microscopic studies were performed according to Clémençon (2009) in the Mycology Laboratory of Trabzon University. In order to view and examine the basidiospores, a piece of basidioma is placed in 3% ammoniated water. After waiting for 2–3 minutes, basidiospores were obtained by tightening with the forceps. To view the structure of pileipellis, sections were taken by hand with a razor blade under a Zeiss Stemi 2000-C microscope, and the sections were mounted in 3% NH<sub>3</sub> solution, stained with aqueous 5% Congo red and examined under a Zeiss Axio Imager A2 trinocular microscope. The images were obtained with the Zeiss AxioCam 105 colour camera, and measurements were made with Imager Software Programme.

### DNA extraction, amplification and sequencing

For the genomic DNA extraction from *Cortinarius* species, a modified protocol based on that of Murray & Thompson (1980) was used. Briefly, the pileus context of the dried specimens was blended in CTAB buffer and mixture was incubated for 15 min at 65°C. After incubation, an equal volume of chloroform: isoamyl alcohol (24: 1) was added and carefully mixed. It was then centrifuged for 10 min at the maximum speed, and the DNA in the supernatant was precipitated by isopropanol. After centrifugation at the same speed, the pellet was washed with cold ethanol, centrifuged again and air-dry pellet re-suspended in ddH<sub>2</sub>O. Molecular phylogenetic analyses of the *Cortinarius* species were performed by sequencing of ITS region. The primers used for the polymerase chain reaction (PCR) were ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al. 1990). PCR reactions were carried out in a 50 µl reaction volume under the following PCR cycling conditions: one cycle of denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 54°C for 30 s, and elongation at 72°C for 45 s and a final step at 72°C for 10 min. PCR products were checked in 1% (w/v) agarose gels, and the PCR products purified following the protocols of the Eco Pure PCR/Gel Purification Kit (ECO TECH Biotechnology, Erzurum, TURKEY). After purification, ITS region was sequenced with one or both PCR primers. Sequences chromatograms were checked searching for putative reading errors, which were corrected.

## Results

### Sequence alignment and phylogenetic analyses

Fig. 1

Sequences (Table 1) were assembled into one complete sequence using Bioedit Sequence Alignment Editor Version 7.2.5 (Hall 1999). The sequences were aligned in MEGA-X software (Kumar et al. 2018) with Clustal W and evolutionary analyses were conducted in same software.

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei 1993) using the standard search algorithm by bootstrap analysis using 1000 replicates. The sequenced PCR amplicons were compared to all known sequences in the GenBank (<http://www.ncbi.nlm.nih.gov/>) by use of BLASTN 2.10.0+ program (Zhang et al. 2000) and UNITE database (<http://unite.ut.ee/>). Sequences of three isolates were deposited in the GenBank database under the accession numbers (MT367144, MT367145 and MT367149).

### **Cortinariaceae R. Heim ex Pouzar**

*Cortinarius cad-aguirrei* Garrido-Ben., Ballarà & Mahiques, Journal des JEC, Journées Européennes du Cortinaire 18(17): 104 (2015) Fig. 2

Index Fungorum number: IF 816060; Facesoffungi number: FoF 09461

Pileus conical to hemispherical, convex, or dome shaped with a low umbo; reddish brown, reddish purple, orange brown, pale brown or reddish orange; margin paler and the centre typically darker; 10–30 mm, hygrophonous, radially striated up to one third of the centre. Lamellae moderately or rather spaced, adnexed, broad, pale brown, yellowish brown or, cinnamon brown; L= 30–40, I= 1–3. Stipe cylindrical, larger towards the base, whitish yellow to cream, watery, 30–60 × 3–5 mm; sometimes the base slightly rounded, fibrous on a pale base, turning pale pink when injured. Universal veil pale cream, scattered in small patches on the stipe surface. Content hygrophonous, pale beige, pale brownish or dark yellowish when dry. Taste and smell indistinct. Basidiospores [n= 55] ellipsoid to slightly amygdaliform, slightly verrucose, granulated, (7.7–)8.5–9.5(–10.5) × (4.8–)4.5–5.8(–6) μm; on average 8.9 × 5.2 μm, Q = 1.4–1.7, Qm = 1.65. Basidia clavate, 4-spored, (29–)30–35(–38) × 7.5–9.1 μm; on average 32.7 × 8.6 μm. Pileipellis consists of incrustated, brownish yellow granulated hyphae. Clamp connections present at all tissues.

Specimen examined – TURKEY, Trabzon, Akçaabat, Fengo plateau, gregarious under spruce (*Picea orientalis* L.) in extreme wetland, 40°55'19.52" N and 39°24'50.46" E, 1638 m alt., 28 Oct. 2019, E. Sesli, KATO Fungi 4148.

*Cortinarius cinnamoviolaceus* M.M.Moser, Nova Hedwigia 14: 514 (1968) Fig. 3

Index Fungorum number: IF 329008; Facesoffungi number: FoF 09462

Pileus convex to campanulate, almost flat, umbonate, striated until halfway to umbo, 30–70 (–80) mm, hygrophonous, chestnut brown to purplish brown or reddish ochre brown, smooth. Lamellae adnate-uncinate to emarginate, sparse, thick, chocolate-brown to light ochre-brown with a purplish tint and smooth; edge whitish, L= 20–25, I= 1–3. Stipe cylindrical, tapering and curved towards the base, 70–120 × 5–10 mm, rigid, fibrillose-striated, whitish, silvery with a purplish tint or slightly violet, typically bright. Content dirty white, slightly violet or purplish. Smell raphanoid or slightly iodine. Guaiac with phenol aniline. Basidiospores [n= 60] ovoid to ellipsoid or tonsilliform, smooth, slightly granulated, (8.2–)9–10.8(11.1) × (4.6–)5–6(–6.7) μm; on average 9.9 × 5.6 μm, Q= 1.6–1.8, Qm= 1.76. Basidia clavate, 4-spored, 35–43 × (7–)8–9(–10) μm; on average 36.3 × 8.6 μm. Marginal cells abundant, filamentous, (15.1–)16.7–26(–29.5) × 6.5–9(–9.5) μm; on average 22.4 × 8 μm. Pileipellis consists of cylindrical to enlarged hyphae. Clamp connections present at all tissues.

Specimen examined– TURKEY, Trabzon, Akçaabat, Fengo plateau, gregarious under spruce (*Picea orientalis* L.), 40°55'22.61" N and 39°25'00.32" E, 1637 m alt., 28 Oct. 2019, E. Sesli, KATO Fungi 4145.

*Cortinarius variegatus* Bres., Fung. Trident. 1(4-5): 56 (1884) Fig. 4

Index Fungorum number: IF 144335; Facesoffungi number: FoF 09463

(Syn. *Cortinarius fulvidolilaceus* P.D. Orton, Notes R. Bot. Gdn Edinb. 29(1): 90 (1969) = *Cortinarius variegatus* var. *marginatus* Bres., Fung. Trident. 1(4-5): 56 (1884) = *Gomphos variegatus* (Bres.) Kuntze, Revis. Gen. Pl.2: 854 (1891) = *Phlegmacium variegatum* (Bres.) Ricken ex Gague, Docums Mycol. 2(8): 3 (1973)

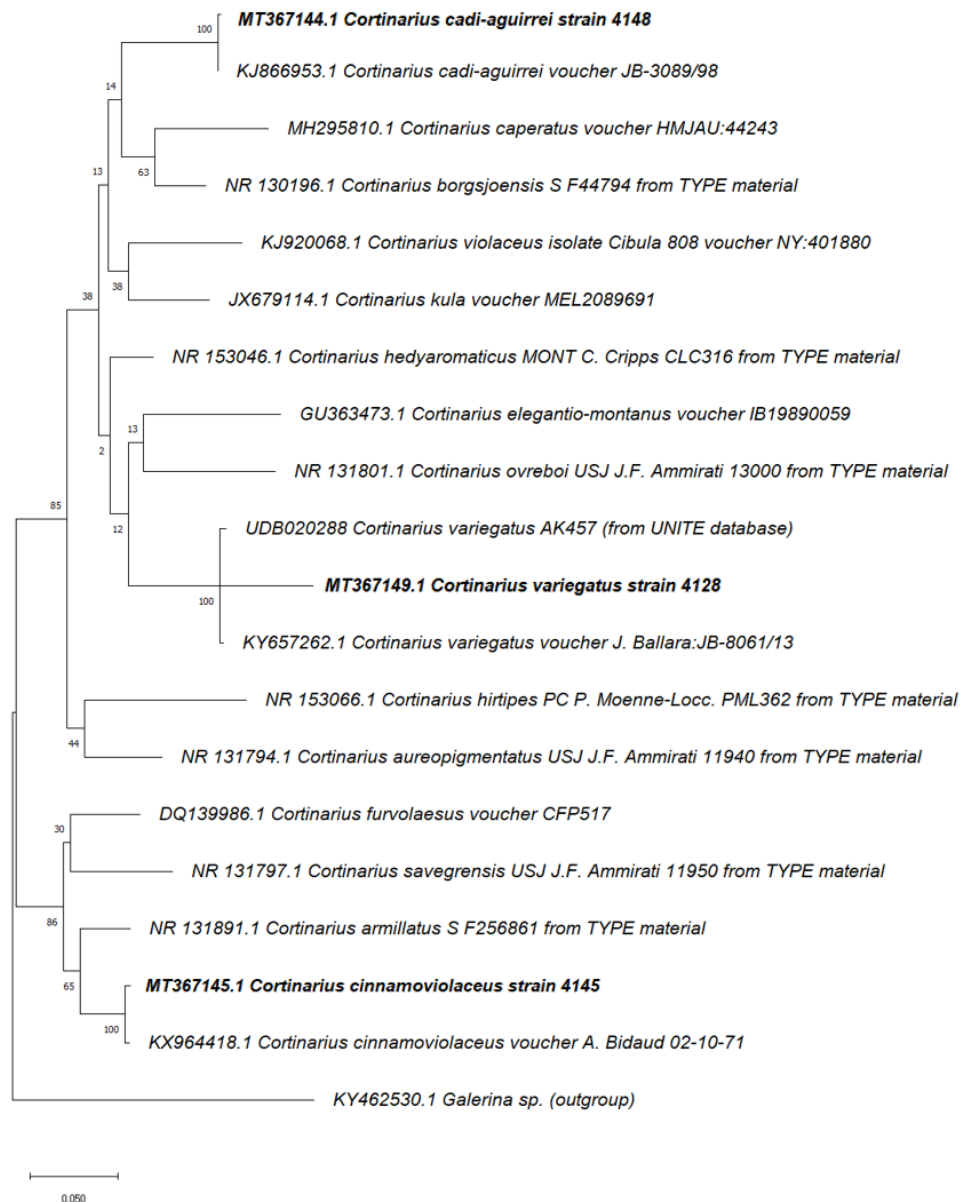
Pileus convex, conical to plane with a broad and larger umbo, fleshy, covered with a slimy gel, fibrillose, surface, excessively sticky, reddish brown, pinkish brown to pale brown, darker at the centre and lighter towards the edge; margin sometimes spitted, 70–100 mm. Content whitish. Lamellae initially whitish, then pale yellowish to cinnamon, darker over time, smooth, adnate to sinuate. Stipe cylindrical, larger towards the base, curved, fibrillose, whitish, subferruginous, marginato-bulbosus, violet to purplish from place to place, covered with a whitish cottony mycelium towards base; 50–70 × 20–30 mm. Basidiospores [n= 50] subamygdaliformis, (6.9–)7.5–9(–10.1) × (2.8–)3–3.8(–4.2) µm; on average 8.5 × 3.5 µm. Basidia clavate, 24–28 × 6.6–7.7(–8) µm; on average 26.1 × 6.7 µm. Pileipellis made up of cylindrical and regular hyphae. Clamp connections present.

Specimen examined– TURKEY, Trabzon, Akçaabat, Fengo plateau, gregarious with *Picea orientalis* L., 40°55'29.39" N and 39°24'29.61" E, 1540 m alt., 28 Sept. 2019, E. Sesli, KATO Fungi 4128.

**Table 1** Taxa names, strains, and GenBank numbers of the taxa used in the phylogenetic analyses

Taxa names	Strain/Voucher	Database	ID	References
<b><i>Cortinarius cadi-aguirrei</i></b>	<b>4148</b>	<b>GenBank</b>	<b>MT367144</b>	<b>This study</b>
<i>C. cadi-aguirrei</i>	JB-3089/98	GenBank	KJ866953	(Garrido-Benavent et al. 2014)
<b><i>C. cinnamoviolaceus</i></b>	<b>4145</b>	<b>GenBank</b>	<b>MT367145</b>	<b>This study</b>
<i>C. cinnamoviolaceus</i>	A. Bidaud 02-10-71	GenBank	KX964418	(Liimatainen et al. 2017)
<b><i>C. variegatus</i></b>	<b>4128</b>	<b>GenBank</b>	<b>MT367149</b>	<b>This study</b>
<i>C. variegatus</i>	J. Ballara: JB-8061/13	GenBank	KY657262	(Ballara et al. 2017)
<i>C. variegatus</i>	AK457	UNITE	UDB020288	-
<i>C. aureopigmentatus</i>	USJ J.F. Ammirati 11940	GenBank	NR_131794	(Ammirati et al. 2007)
<i>C. savegrensis</i>	USJ J.F. Ammirati 11950	GenBank	NR_131797	(Ammirati et al. 2007)
<i>C. ovreboi</i>	USJ J.F. Ammirati 13000	GenBank	NR_131801	(Ammirati et al. 2007)
<i>C. borgsjoensis</i>	S F44794	GenBank	NR_130196	(Liimatainen et al. 2014)
<i>C. hedyaromaticus</i>	MONT C. Cripps CLC316	GenBank	NR_153046	(Cripps et al. 2015)
<i>C. hirtipes</i>	PC P. Moenne-Locc. PML362	GenBank	NR_153066	(Niskanen et al. 2016)
<i>C. elegantio-montanus</i>	IB19890059	GenBank	GU363473	(Garnica et al. 2011)
<i>C. furvolaesus</i>	CFP517	GenBank	DQ139986	(Kytovuori et al. 2005)
<i>C. caperatus</i>	HMJAU: 44243	GenBank	MH295810	-
<i>C. armillatus</i>	S F256861	GenBank	NR_131891	(Kytovuori et al. 2005)
<i>C. kula</i>	MEL2089691	GenBank	JX679114	(Stefani et al. 2014)
<i>C. violaceus</i>	NY: 401880	GenBank	KJ920068	(Harrower et al. 2015)

\* Sequences produced from this study are in **black bold**



**Fig. 1** – Phylogenetic relationship of present study ITS sequences by Maximum Likelihood method. The tree with the highest log likelihood (-4511.53) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 20 nucleotide sequences. There were a total of 834 positions in the final dataset. *Galerina* sp. was used as outgroup.

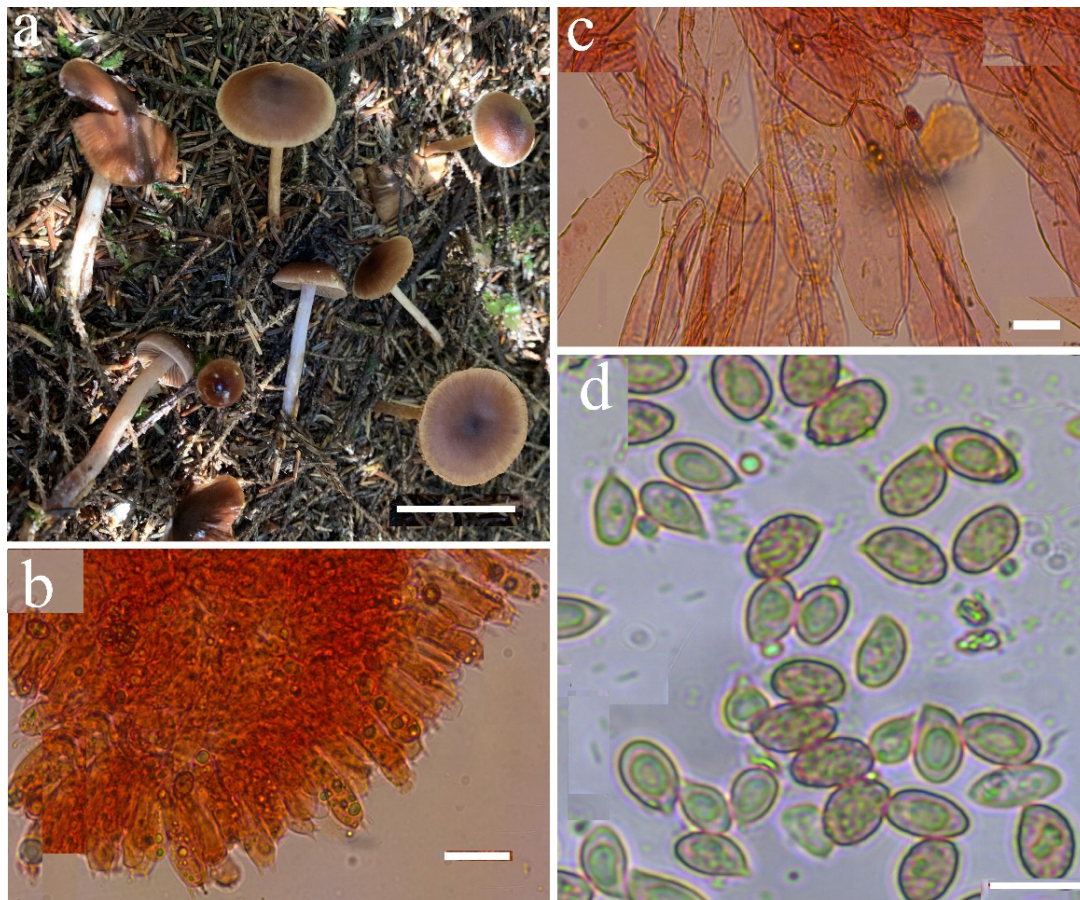
### Key to *Cortinarius* species

- 1. Basidiospores 8 – 9  $\mu$ m .....2
- 1` Basidiospores larger than 9  $\mu$ m .....2`
- 2. Lamellae adnate to sinuate .....*Cortinarius variegatus*
- 2` Lamellae different .....3
- 3. Basidia medium sized .....*Cortinarius cadi-aguirrei*
- 3` Basidia longer .....*Cortinarius cinnamoviolaceus*

## Discussion

*Cortinarius cadi-aguirrei*, *C. cinnamoviolaceus* and *C. variegatus* are recorded for the first time from Turkey and provided here with descriptions, field and microscopic photos and a phylogenetic tree. Our results about the new records generally matched very well with the original descriptions. Findings connected with *Cortinarius cadi-aguirrei* by Garrido-Benavent et al. (2015) (10–40 mm, orange, reddish orange to apricot-like orange pileus, 40–70 × 4–7 mm, pale brownish stipe, 8–10.5 × 4.5–5.5 μm, mostly ellipsoid, sometimes slightly amygdaliform basidiospores) fit well with KATO Fungi 4148.

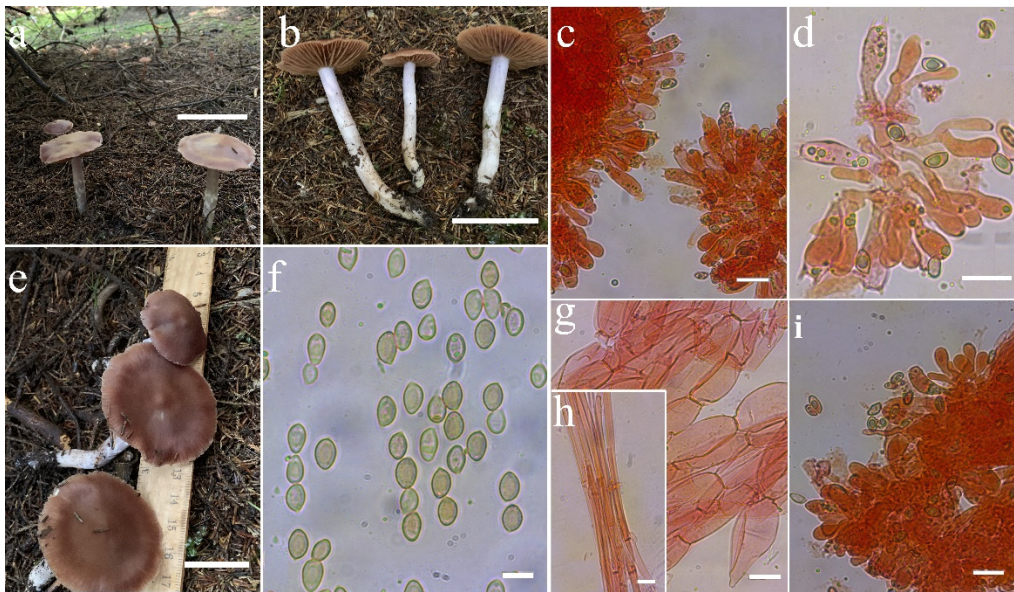
Another species collected from Trabzon (KATO Fungi 4145) is the same with *Cortinarius cinnamoviolaceus* described by Bellanger et al. (2017). According to the other morphological and molecular evaluations, we think that the reason for some small differences are due to the different ecological conditions.



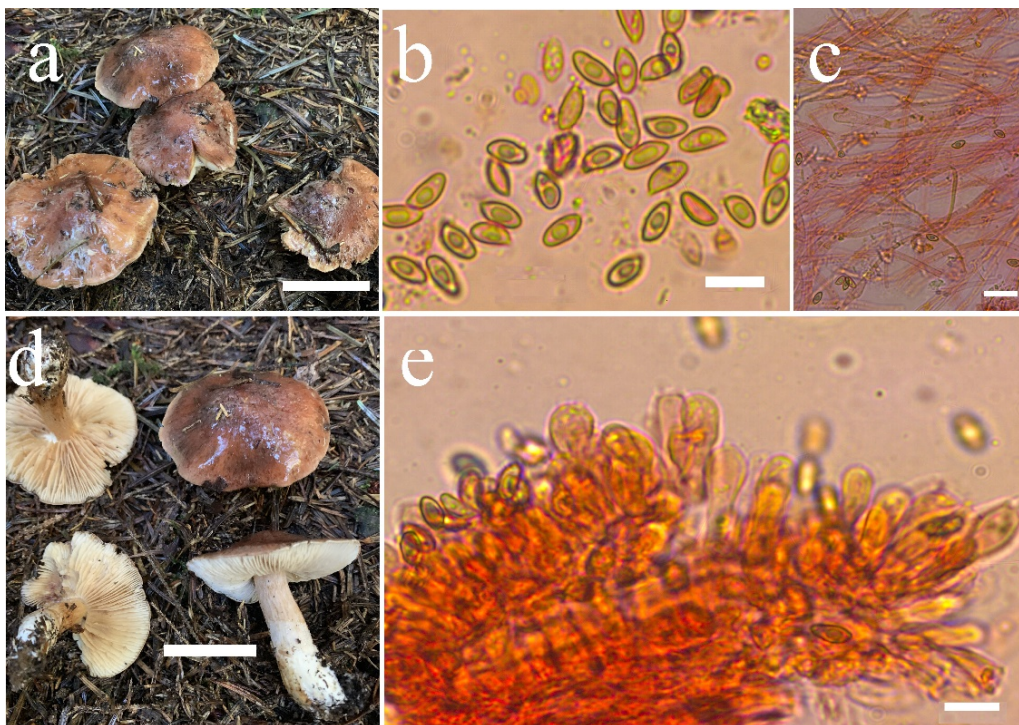
**Fig. 2** – *Cortinarius cadi-aguirrei*. a basidiomata. b basidia. c pileipellis. d basidiospores. Scale bars: a = 30 mm, b, c = 20 μm, d = 10 μm.

*Cortinarius variegatus* described by Bresadola (1884) is highly compatible with our findings (KATO Fungi 4128). The molecular characterization was performed by carrying out sequencing of rDNA ITS region. In this study, the ITS region (ITS1-5.8S-ITS2) amplified with ITS1 and ITS4 pair of primers varied in length from 580 to 618 bp in the three species. The ITS sequences were compared to all known sequences in the GenBank for confirmation of their identity. Based on percentage identity and query coverage, the three *Cortinarius* species identified as follows: accession MT367144 showed 99% identity with *C. cadi-aguirrei* (KJ866953.1; 100% coverage); MT367145 showed 99% identity with *C. cinnamoviolaceus* (KX964418.1; 99% coverage) and MT367149 showed 97% 99% identity with *C. variegatus* (KY657262.1; 92% coverage, UDB020288; 99% coverage respectively). These three species were identified up to species level from the GenBank database. In this study, we used a combined approach with morphological and

molecular analysis of *Cortinarius* species. This approach has proven very useful and valuable tool for correct identification. Some authors have also used ITSr DNA sequences combination with morphological characters for identification of *Cortinarius* species (Peintner et al. 2003, Becerra et al. 2005, Ito et al. 2015, Sesli & Liimatainen 2018, Şengül Demirak et al. 2020).



**Fig. 3** – *Cortinarius cinnamoviolaceus*. a, b, e basidiomata. c, d, i basidia and marginal cells. g, h pileipellis. Scale bars: a, b = 70 mm, e = 35 mm, c, d, g, i = 20  $\mu$ m, h = 10  $\mu$ m.



**Fig. 4** – *Cortinarius variegatus*. a, d basidiomata. b basidiospores. c pileipellis. e basidia. Scale bars: a, d = 50 mm, b, e = 10  $\mu$ m, c = 20  $\mu$ m.

Currently, 15843 records in the GenBank and 2064 records in the UNITE database are available for *Cortinarius* genus. As the available data increases, it is more possible to solve identification in the *Cortinarius* genus (Şengül Demirak et al. 2020). Our ITSr DNA data may contribute to the correct identification of *Cortinarius* species and related taxa.

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