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Notes on four mediterranean Cortinarius fruiting in sclerophilous and heliophilous plant ecosystems

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Abstract — Four mediterranean Cortinarius (subgenus Telamonia) taxa associated with oaks and pure Cistus stands are described and discussed. New type material of C. contui is designated, and the type material of C. bulbosovolvatus is confirmed. The new taxon C. assiduus var. plesiocistus is proposed. Micrographs (including those of holotypes) and FESEM photographs of the spores, as well as photographs of the basidiomes in their habitat are included. Analysis of nucleotide sequences (ITS region) is afforded.

Key words - Cortinariaceae, Quercus, Spain, Europe

Introduction

According to our observations, two of the four taxa studied in the present work (Cortinarius bulbosovolvatus and C. contui) grow exclusively under Cistus sp. on acid soil. The other two taxa are varieties of C. assiduus: the typical variety, which fruits on basic soil under both Quercus ilex and Cistus, and is recognized by the scarcely developed universal veil, and var. plesiocistus (described in the present work), which is a exclusive Cistus monspeliensis associate that grows especially on acid soil and exhibits a conspicuous veil.

C. contui is a species that has been often misinterpreted by Spanish (Ortega & Mahiques 1995, Vila 2002, Vila & Llimona 2002, Torrejón 2003) as well as Italian (Contu 1991) mycologists, who have identified as C. contui several

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collections characterized by fasciculate habit, a hardly hygrophanous pileus with abundant white veil also present on the stipe, and broadly ellipsoid spores, a combination of features which is reminiscent of *C. bulbosovolvatus* and, above all, *C. assiduus* s.l. Since the original material of *C. contui* is missing and no isotype was designated by its authors (Henry & Contu 1987), we think it is necessary to designate a neotype based on material conserved at CAG (University of Cagliari, Sardinia).

C. bulbosovolvatus grows in similar ecosystems and is recognized by its general appearance, which brings to mind a small C. bulbosus (Sowerby) Fr. (Henry & Contu 1985, Phillips 1980, Soop 2005). However, the latter has a distinct volva and different spores and habitat. Part of the original material of C. bulbosovolvatus is kept at PC (the herbarium label states Cortinarius sp., while the collection number is the same as that reported by Henry & Contu 1985). Notwithstanding, its deficient conservation prevented us from confirming the identity.

Some Cistus monspeliensis-associated forms of C. assiduus exhibit characters that permit a separation from these fruiting in woods, especially by the less hygrophanous pileus, the fasciculate habit and abundant veil. These forms are frequently found in France, where it has been known as C. cistoadelphus nom. prov. (Bidaud 1994).

The present work focuses on the study of the types of the above taxa, as well of other material from Italy and the Iberian Peninsula, with the purpose of establishing a concept for each taxon and its taxonomic circumscription.

Material and methods

We have studied the type material of *C. assiduus* var. assiduus (MES-3541, R. Mahiques private herbarium), *C. bulbosovolvatus* (CAG, herb. Istituto Orto Botanico, Cagliari; PC, herb. Museum National d'Histoire Naturelle, Paris), *C. cistoadelphus* (AB. N° 92-11-422, A. Bidaud private herbarium) and *C. contui* (CAG); as well as other collections of these species from Italy (Sardinia; CAG) and Spain (Andalusia, Catalonia, Valencian Community: GDAC-GDA; JA-Cussta; MES; JVG, J. Vila private herb.; MTH, M. Torrejón private herb.). Basically, the bibliography that refers to these taxa includes Henry & Contu (1985, 1987) and Mahiques et al. (2001). The morphological study of spores has been carried out by means of a Zeiss scanning electron microscope of the FESEM type (Field Electron Scanning Electron Microscope), model 1539 Geminis. Sporal data include mean spore size (length, width, and L/W ratio), from 30 spores of each collection. Code of colour: Kornerup & Wanscher (1973). The analysis of DNA sequences of *C. assiduus* var. assiduus and *C. assiduus* var. plesiocistus has been made by Dr. S. Garnica (University of Tübingen, Germany).

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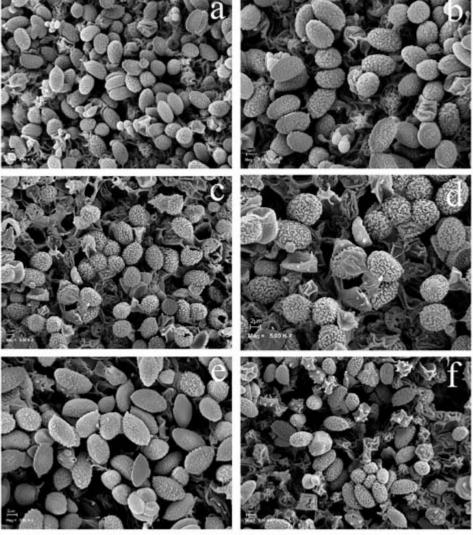


Plate 1.- Spores under FESEM microscope: a-b) Cortinarius assiduus var. piesiocistus (holotype); c-d) C. contui (neotype); e-f) C. bulbosovolvatus (holotype and CAG890108/2).

Results

Cortinarius assiduus Mahiques, A. Ortega & Bidaud var. assiduus, Bull. Féd. Mycol. Dauphiné-Savoie 41(162): 42 (2001)

SPECIMENS EXAMINED — SPAIN. GRANADA: Cortijos of Valparaiso (La Alcaicería), under *Quercus ilex* subsp. *ballota* and *Cistus laurifolius*, in decarbonated soil, 14.12.2005, GDA 52898.- Wood path of las Pajareras, km 0.5 (natural park of the Sierra de Huétor, Huétor, Santillán), under *Q. ilex* subsp. *ballota*, in decarbonated soil, 13.11.2006, leg. A. Ortega, GDA 53896.- Ibid., 26.11.2006, GDA 52897. Jaén: Castañeda, near the Quiebrajano reservoir, under *Q. ilex* subsp. *ballota*, in calcareous soil, 1.1.1987, GDAC

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36735.- Cuesta Grande (Santiago, Pontones), 1350 m, under Q. ilex subsp. ballota, in calcareous soil, 25.10.2002, leg. J.D. Reyes, JA-Cussta 2150.- Castro Ferraz (Santa Elena), 900 m, under Q. ilex subsp. ballota, Q. suber, C. ladanifer and Pinus pinea, in decarbonate soil, 27.11.2003, leg. J.D. Reyes, JA-Cussta 3296. TARRAGONA: Vilanova de Prades (Conca de Barberà), Corral del Sanç, 1005 m, under C. laurifolius, in acid soil, 16.4.2002, leg. X. Llimona, J. Vila, J. Llistosella & C. Gutiérrez, JVG 1020416-24.- Prades (Baix Camp), road to Albarca, 910 m, under C. albidus y Q. ilex subsp. ballota, in basic soil, 16.4.2002, leg. X. Llimona, J. Vila, J. Llistosella & C. Gutiérrez, JVG 1020416-12 (EMBL accession number AM 713179). VALENCIA: Els Surars (Pinet), 660 m, under Q. suber and P. pinaster, in sandy soil, 11.11.1999, leg. R. Mahiques, MES 3541 (holotypus).-Ibid., MES 3359, 3363, 3364, 3541.

Discussion: See C. assiduus var. plesiocistus.

Cortinarius assiduus var. plesiocistus A. Ortega, Vila & Bidaud var. nov.

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A typo differt sporibus maioris, cuticula leviter hygrophana et habitat stricto inter Cistum monspeliensem.

Holotypus: prope vico Cadaques dicto, in NE Catalonia (Hispania), a X. Llimona et J. Vila lectus, 25-1-1999, 40 m altitudinis, sub Cisto monspeliensi, in herb. GDA 52535, Universidad de Granada conservatus (isotypus in JVG 990125-31).

Etymology: an adjective, from the Greek plesios = neighbouring, and Kistus (Cistus).

Basidiomes normally in small groups of 3-4 specimens, more rarely solitary. Pileus 20-50 mm in diam., at first hemispherical to convex, then conicoapplanate, later expanded from plano-convex to applanate, occasionally with a low, blunt umbo, not depressed; margin incurved when young, then straight, normally smooth but in some specimens slightly sulcate. Universal veil abundant, whitish, later with a silvery sheen, fibrillose, forming rather persistent patches, especially at the disc, hardly present along the margin; some collections with reduced veil to some marginal fibrils. Surface shiny, sometimes more or less viscid, hardly hygrophanous (with small scattered hygrophanous spots), reddish-brown (7C-8), generally darker, especially at the disc, somewhat much paler at the margin, often with vinaceous (9C-4, 9E-8, 10D-5) or violaceous (8 C-3) tinges. Lamellae relatively thick, slight to moderately crowded, adnate to adnate-sinuate or decurrent by a tooth, brown (6E-8) or rusty brown (6D-8) with more or less lilac (10C-3) tinges; edge even and concolorous. Lamellulae numerous. Stipe 30-60 × 10-20 mm, rather thick-set, sometimes curved, base terete or slightly enlarged, white, rarely with brownish spots, surface fibrillose. Cortina white, copious, becoming rusty due to the spores. Context firm, whitish with violaceous (8C-3) tinges, especially in stipe apex; odour and taste subraphanoid.

Spores (7.5–)8.8–10(–11.2) × (4.6–)5.2–5.6(–6.5) μ m, ellipsoid to narrowly ellipsoid (Q: L/w = (1.43–)1.55–1.9(–2.1), ochraceous-brown (OM), with slight to moderately sized, sparse warts. Basidia up to 30 × 10 μ m, clavate

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Plate 2.- Habitus: a) Cortinarius assiduus var. plesiocistus; c-d) C. contui.

to subcylindrical, 4-spored, with basal clamps. Marginal sterile cells hardly differentiated. Suprapellis composed by a thin layer of narrow, cylindrical hyphae, with numerous colourless or slightly pigmented terminal elements, below which the hyphae are more packed and exhibit a mixed pigmentation: intracellular and incrusting epiparietal, brownish or reddish-brown. Mediopellis

cellular, with chains of short, broad, clearly differentiated cells, colourless or slightly pigmented in the upper layers. Subpellis subcellular.

Molecular analysis: analysis of nucleotide sequences (ITS region) indicates a six base pair difference between C. assiduus var. assiduus and C. assiduus var. plesiocistus.

SPECIMENS EXAMINED — SPAIN. CASTELLÓN: Mas Blanc (Benicasim), natural park of las Palmas Desert, 170 m, under Cistus monspeliensis and C. salviifolius, in acid soil, 27.11.2001, leg. M. Torrejón, MTH 219.- Ibid., MTH 226. GIRONA: Roses (Alt Empordà), plains between Can Marés and Canyelles Petites, 130 m, under C. monspeliensis and C. albidus, in acid soil, 25.1.1999, leg. J. Vila & X. Llimona, JVG 990125-50.- Roses (Alt Empordà), la Falconera, near the road to the abandoned military installations, 95 m, under C. monspeliensis, C. salviifolius and C. albidus, in acid soil, 23.1.2001, leg. J. Vila & X. Llimona, JVG 1010123-12.- Cadaqués (Alt Empordà), Cala Jonquet, 40 m, under C. monspeliensis, in acid soil, 25.1.1999, leg. J. Vila & X. Llimona, GDA 52535 (holotypus), JVG 990125-31 (isotypus) (EMBL accession number AM 713178).- Ibid., 1.2.2000, leg. J. Vila & X. Llimona, JVG 1000201-14.

Discussion: C. assiduus s.l. is a mediterranean species close to C. saturninus (Fr.) Fr., exhibiting an ample variability and different ecological preferences. It may, in fact, grow in sclerophilous woods of Quercus ilex subsp. ballota, Q. coccifera, Q. suber, both in acid and basic soil, as well as in Cistus formations, in some cases thermophilous (thermo-mediterranean belt) and in others more mesophilous (meso- and supra-mediterranean belts), where it may establish relationships with several species (C. monspeliensis, C. salviifolius, C. ladanifer and C. laurifolius among others).

The populations fruiting in association with oaks are often characterized by strongly hygrophanous basidiomes and show bluish-violaceous or bluish-lilacineous tinges on lamellae, upper stipe, context and even on the pileus surface. The development of the universal veil is scanty and ephemeral and the spores are on average smaller, 7.8–9.5 × 4.6–4.9 μm, than those observed in the collections associated with *Cistus*. In acidophilous *Cistus monspeliensis* formations, especially of Catalonia and Valencia, we have observed populations rather different from the former, as basidiomes generally are smaller, less hygrophanous (hygrophanity irregular, in patches), the bluish-violaceous tinges are hardly visible, the universal veil has a stronger and lasting development (feature observed in many *Cistus* associates), and the spores are larger, 8.8–10 × 5.2–5.6 μm. We refer to these collections as *C. assiduus* var. plesiocistus. The analysis of DNA sequences prove the differences between the *Quercus* (sometimes with *Cistus laurifolius* or *C. ladanifer*) and *Cistus monspeliensis* populations of *C. assiduus*.

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Table 1. Herbarium collections examined.

Herb	Coll. number / Status	Original name	Final disposition
CAG	860119/01 (duplicate holotype)	C. contui	C. assiduus var. plesiocistus & C. bulbosovolvatus
CAG	840127/01 (holotype)	C. bulbosovolvatus	C. bulbosovolvatus & C. contui
CAG	890108/02 (part of PC 0090926)	C. contui	C. bulbosovolvatus
Hry	nº84/159 (isotype bulbosovolvatus)	unnamed	damaged (C. bulbosovolvatus?)
PC	0090926 (RH and C 89)	unnamed	C. bulbosovolvatus & C. contui
Hry	nº86/47 (PC) (holotype)	C. contui	destroyed

Cortinarius bulbosovolvatus Rob. Henry & Contu, Doc. mycol. 16 (61): 32 (1985)

ORIGINAL DESCRIPTION— Pileo (15)–30–45–(50) mm, sat carnoso, primo regulari subhemisphaerico, demun plano ad saepe irregulari, rare umbonato, hygrophano, glabro, mudo, sublucente; cute humida luteo-brunnea vel obscure brunnea, ad medium subnigra; margine pellucido-striata, sicca pallidiore, separabili. Lamellis haud confertis, sat spissis, inaequalibus, adnato-decurrentibus, ex argillaceis fusco-castaneis, acis integris; raro denticulatis. Stipite (30)–40–45–(50) mm alto, apice 5–10 mm spisso, non vero elato, sat firmo, cylindrico sed ad basim constanter bulboso volvaque alba conspicua praedito fragili; ceterum indumento sericeo fibrilloso induto saepe diffracto; sursum deorsumque albo, in medio subbrunneo, residuis brunneis cortinae decorato, mycelio albo. Carne firma, fragili, ex albida pallide cinereo-brunnea, sub cute flavobrunnea, odore nullo, sabore debili interdum acidulo. Cortina copiosa fibrillosa alba in juventute valde conspicua. Sporis ellipsoideo-ovoideis vel anguste, amygdaliformibus, acute apiculatis, verrucosis (8.7–10.8 × 4.3–5 µm). Basidis 4-sp. (29–36 × 7.2 µm). Pilis sterilibus (15 × 7.2 µm). Gregarius saepe caespitosus (5-6 sp), in Cistitis sabulosis acidiphilisque, praecipus sub C. monspeliensis. Vere autommoque, sat frequens.

SPECIMENS EXAMINED— ITALY. CAGLIARI: surroundings of Maracalagonis, locality Pixina Nuxedda, under Cistus monspeliensis, in acid soil, 27.1.1984, leg. M. Contu & L. Curreli, CAG 840127/01 (holotypus), herb. R. Henry 84/159 (isotypus).- Maracalagonis, Pixina Nuxedda, under Cistus, 8.1.1989, leg. M. Contu & V. Nieddu, CAG 890108/02 (as C. contui), herb. R. Henry (RH) and C 89, PC 0090926 (as Cortinarius sp.). SPAIN. GIRONA: Cadaqués (Alt Empordà), Cala Jonquet, 40 m, under C. monspeliensis, a single specimen, in acid soil, 25.1.1999, leg. J. Vila & X. Llimona, JVG 990125-30.

Discussion: The collection CAG 840127/01 (holotypus), of which we have studied two specimens, is in good condition. One of the specimens matches

Cortinarius bulbosovolvatus very well, with a pileus of 25 mm in diameter and a stipe of 45 × 5 mm, with the base sheathed in a visible fibrous-membranous volva (less evident than in collections CAG 890108/02 and PC 0090926). The spores measure $(8-)9.2-9.7(-11.2) \times (4.8-)5.2-5.3(-6) \mu m$, are ellipsoid to subcylindrical (Q: L/w = (1.5-)1.78-1.83(-2)) and have a slight to moderate ornamentation, consisting of more or less coalescing warts. The second specimen studied is quite different; there is no volva and the spores are significantly larger, $(9-)10.1-10.3(-11) \times (5.5-)5.9-6.1(-6.8)$ µm; for this reason we think it might represent C. contui. The voucher CAG 980108/02 (labelled C. contui) consists of seven basidiomes, pileus 24-40 mm in diameter, stipe 30-45 × 8 mm (base 12-14 mm), sheathed in an conspicuous volva, spores (8.8-)9.5-10(-11) × (4.8-)5.2-5.4(-5.7) μm, ellipsoid to almost cylindrical (Q: L/w = (1.6-)1.8-2(-2.2)), with a slight to moderate ornamentation, consisting of more or less coalescing warts and a suprapellis with hyphae 1.5-3 µm wide, arranged in fascicles forming a loose surface layer with numerous free cylindrical terminal elements, under which there is another layer with compact hyphae; the mediopellis is composed of parallel hyphae with variable wide (5-6 μm, 10-18 μm, or 10-24 μm) forming several layers, the deeper ones with moniliform elements; pigment cellular, ochraceous-yellowish, basically localized in the subpellis hyphae; subpellis cellular. This collection is certainly to be ascribed to C. bulbosovolvatus and matches most of the specimens in collection PC 0090926.

The collection Hry n° 84/159 (not labelled), which is the isotype, is poorly preserved so that it was possible to study only the spores: $8.4-10 \times 5-6 \mu m$. In the same envelope, there is also additional unnamed material (three specimens) with the references RH and C 89, PC 0090926. Two of the specimens have a cap of 28-40 mm in diam., stipe $31 \times 5 \text{ mm}$, with a conspicuous volva and spores $(8-)9.1-9.2(-10) \times (4.8-)5.1-5.3(-5.7) \mu m$, ellipsoid (Q: L/w = (1.5-)1.7-1.8(-2)), with a morphology and ornamentation similar to that of the other collection (Hry n° 84/159). In our opinion they fit perfectly the concept of *C. bulbosovolvatus*. The third specimen is much smaller, has no volva and its spores are different, $8.9-10.8 \times 5.3-6.1 \mu m$, and we think it might be ascribed to *C. contui*.

So far, C. bulbosovolvatus was known only from Sardinia (Italy) and was confused with other Cistus-associated taxa. Its separation from C. contui and C. assiduus s.l. is not difficult due to its macro- and microscopic features: presence of a distinct white volva, which remains visible even in herbarium material, and the pileipellis lacking the pseudoparenchymatic layer occurring in the other species. It deserves to be pointed out that the observed spore size is much more variable than noted in the original description.

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Cortinarius contui Rob. Henry & Contu, Doc. Mycol. 17 (68): 41 (1987), non C. contui sensu Contu (1991), non C. damascenus subsp. contui sensu Ortega & Mahiques (1995).

Neotypus (designated here): ITALY. CAGLIARI: Foresta Demaniale dei Sette Fratelli, loc. Monte Cresia, in a pure *Cistus* formation with *C. monspeliensis* and *C. salviifolius*, in acid, sandy soil, leg. M. Contu, det. R. Henry & M. Contu, M.C. 86/02 (CAG).

Basidiomes growing in fascicles of 5-6 specimens sharing a common base which is distinctly enlarged and sheathed in a copious mycelium with remnants of substrate. Pileus 25-65 mm in diam., hemispherical-convex, convex or planoconvex with a central depression in fully expanded specimens, rarely with a low rounded umbo; margin at first incurved, then straight, regular; universal veil hardly developed, even though some whitish fibrillose remnants can be observed both on the disk and along the margin. Surface smooth, shiny, hygrophanous, reddish-brown (8E-8), but soon drying to orange-brown (7C-8, 7C-4); herbarium material showing a yellowish-brown colour (5E-6) with greyish-orange tinges (5B-3), brownish-orange (5C-3) or Sahara (6C-5). Lamellae thick, moderate crowded, adnate-sinuate or adnate-subdecurrent, reddish-brown (8C-8, 8D-8). Stipe 35-65 × 7-14 mm, rather firm, thick-set and rigid, cylindrical or with slightly enlarged base, very dark (7C-8) with age or when handled; surface smooth. Herbarium material shows fibrillosemembranous remnants of the universal veil, which may form a more or less distinct volvate structure. Cortina white, then brownish, copious over the stipe surface. Context firm, whitish but pale browning; odour and taste raphanoid.

Spores $(7.2-)8.6-10.5(-11.2) \times (5-)5.6-6.6(-7.2)$ µm, very variable in morphology: amygdaliform, ellipsoid, broadly ellipsoid even subglobose (Q: L/w=(1.26-)1.37-1.7(-1.77)), ochraceous-yellowish to ochraceous-brown (OM), with moderate ornamentation, consisting of more or less coalescing, isolated or anastomosed warts. Basidia $20-28\times7-8$ µm, cylindric-clavate, hyaline or with ochraceous vacuolar content, 4-spored. Marginal sterile cells $13-16\times7-8$ µm, similar to basidia, clavate or subpyriform. Pileipellis differentiated. Suprapellis a loose layer of yellowish hyphae, 4-6 µm wide, with numerous free, cylindrical terminal elements. Mediopellis pseudoparenchymatic, with compacted hyphae, some 7-9 µm wide, others 8-12 µm, rarely up to 25 µm, with septa so numerous as to give it a cellular appearance. Subpellis hardly differentiated, cellular. Pigment intracellular, but also slightly incrusting in some superficial hyphae.

SPECIMENS EXAMINED— ITALY. CAGLIARI: Foresta Demaniale dei Sette Fratelli, loc. Monte Cresia, in a pure *Cistus* formation with *C. monspeliensis* and *C. salviifolius*, in acid, sandy soil, leg. M. Contu, det. R. Henry & M. Contu, M.C. 86/02 (CAG, neotypus). SPAIN. GIRONA: Roses (Alt Empordà), Coll de la Perafita, 250 m, under *C. monspeliensis*, thickly gregarious, in acid soil, 28.11.2000, leg. J. Vila & X. Llimona, JVG 1001128-16, GDA 52536.

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Discussion: This species fruits exclusively in association with Cistus in acid soil; however, it does not seem to be as frequent as claimed by Contu (1991) and Henry & Contu (1987). We think it likely that it has been confused with C. assiduus s.l. (C. castaneus sensu Konrad & Maublanc, Malençon & Bertault) and with C. bulbosovolvatus. As pointed out above, the material designated as type by Henry & Contu (1987) is missing (Henry's herb. n. 86/47 in PC 0090925). Therefore, we obtained information from CAG, where many taxa coming from the same locality as C. contui are deposited, that the herbarium holds three collections labeled C. contui. The first one (CAG 860119/01), labeled "duplicato dell'holotypus (herb. R. Henry/Vesoul)", includes several specimens; of the specimens studied exhibit characters which do not fit the concept of C. contui, one is to be ascribed to C. assiduus, the other, most likely, to C. bulbosovolvatus. The second, CAG 890108/02, of which we have studied two specimens and photographed the whole collection, matches perfectly C. bulbosovolvatus. Finally, the third (M.C. 86/02), including a single basidiome which we have studied and photographed, fits perfectly the concept of C. contui. Accordingly, we propose it as a neotype. We think that, according to the comments of Contu (1991) about the holotype of this species, and on the basis of both its features and microanatomy, the material labelled CAG 860119/01 corresponds to C. assiduus, which would substantiate the hypothesis by Henry & Contu (1987) that C. contui may also grow under oaks.

The relationship between *C. contui* and *C. damascenus* Fr. appears obvious; the fasciculate growth, a similar appearance and pileipellis structure. They separate by the abruptly tapered stipe base (it brings to mind a pencil) and the much smaller spores (rarely over 9 x 5.5 μm) of *C. damascenus*, as reported by many authors (Brandrud 1992, Breitenbach & Kränzlin 2000, Henry 1983, Herzog 2001, Horak 2005, Marchand 1983).

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