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***Cortinarius xanthodryophilus* sp. nov. – a common *Phlegmacium* under oaks in California**

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ABSTRACT -- A new *Cortinarius* species (subgenus *Phlegmacium*) associated with oaks in California is described. *Cortinarius xanthodryophilus* is a commonly encountered representative of the fulvoid bulbopodiums and is characterized by predominantly light to dark yellow colors, distinctly emarginated bulb, weak alkali reaction, and association with oaks. Based on phylogenetic analysis of nrITS sequences, it is placed in the /pseudoglaucopodes/humolens clade.

KEY WORDS -- *Cortinariaceae*, fungal taxonomy

Introduction

The state of California, USA spans several distinct ecological regions with diverse biota and climatic patterns. The genus *Cortinarius* is widespread throughout the state with primary symbionts ranging from conifers along the Northern coastal belt and the Sierra/Cascade mountain ranges to hardwoods in the warmer coastal zone and the xeric Mediterranean-type climate areas.

Historically, the major contributions to the study of the genus in the state were initiated by A. H. Smith, who described a number of taxa from the Pacific Northwest and California (Smith 1939, 1944; Smith & Rea 1944). Ammirati, who studied *Dermocybe*, also published new species from the area (Ammirati & Gilliam 1975, Ammirati & Smith 1977, 1978, 1984). In the 1990's, Moser and Ammirati, who published a series of studies on Pacific Northwest taxa, described a number of *Cortinarius* species from California and surrounding areas (Moser 2002; Moser & Ammirati 1996, 1997, 1999, 2000). Thiers & Smith (1969) and Fogel (1994) made important contributions to the study of hypogeous cortinarii in the region, and a few *Cortinarius* taxa have been described from California by other authors. Environmental sampling is becoming an important tool for detecting *Cortinarius* species from root tips that have yet to be collected or reported in the literature.

During our study of *Cortinarius* in California for the past six years, we have collected a number of undescribed taxa. The description of *Cortinarius xanthodryophilus* is our first installment in a planned series of contributions to the study of *Cortinarius* in California and the Pacific Northwest.

Materials & methods

MORPHOLOGICAL STUDIES: All collections have been observed and described by the authors. Emphasis was placed on describing basidiomata in all developmental stages in order to analyze such fleeting characters as the original lamellar/context colors and bruising reactions. All fresh material was processed on the day of the collection. In all cases we tested the fresh material for odor, taste, macrochemical reactions, and UV reflection. High-resolution photographs with bracketed exposures (+1, 0, -1) EV were made for each fresh collection. Taxonomically important macromorphological features were carefully depicted. High-resolution photographs are available online (see www.mushroomhobby.com under genus *Cortinarius*). $\text{KOH}_{5\%}$ and Melzer's were the primary agents for macrochemical analysis in *Cortinarius* taxonomy. KOH was applied to all external surfaces and context of the pileus, stipe and bulb. Color codes follow the Munsell soil color charts (2000). Spore prints were collected directly on microscopic slides in order to evaluate the precise shade of the spore color and to obtain a rich set of mature spores for study. UV fluorescence was tested with a 400 nM Ultra Violet Blacklight Flashlight. All examined collections are preserved in the first author's private herbarium, designated as DBB, and (where noted) in the University of California, Berkeley Herbarium (UC).

MICROSCOPIC STUDIES were conducted using a light compound microscope. Basidiospores (aberrant spores excluded) were studied at magnifications of 1000–1600 \times under immersion oil, with two slides with spores from two different basidiomata examined for each collection. A minimum of 32 spores were measured in each case. The spores were mounted in H_2O , KOH and Melzer's reagents. The following abbreviations are used: Q for quotient of length and width and Q_{av} for average quotient. Cell structures were studied at 640–1600 \times magnification and observed in KOH, Melzer's, and H_2O . Congo Red was used for the study of cuticular morphology, cell structures, cell walls, and incrustations. All microstructure measurements reflect data from all examined collections, including the holotype.

MOLECULAR EXTRACTION: The universal primers, ITS4 and ITS5, were used to amplify the internal transcribed regions (ITS 1, ITS 2), the 5.8 gene nuclear ribosomal subunit, and part of the large and small ribosomal subunits by polymerase chain reaction (White et al. 1990). Amplification was carried out in 50 μl reactions containing 3 μl DNA, 50 mM KCl, 10 mM Tris-HCl (pH 9), 1% Triton X-100, 2.5 mM each of dATP, dCTP, dGTP, and dTTP, 25 mM MgCl_2 , 50 mM of each primer, 1 unit of *Taq* polymerase, and 32.6 μl of milliQ water. The PCR reaction was conducted using a PTC-100 thermocycler (MJ Research, Watertown, MA) with the following parameters: 40 cycles of 1 min at 94 $^\circ\text{C}$, 2 min at 55 $^\circ\text{C}$, and 2.5 min at 72 $^\circ\text{C}$, and a final extension time of 10 min at 72 $^\circ\text{C}$. Negative controls (no template DNA) were included in every assay. PCR-amplified DNA was visualized on 1.5% agarose gels (Invitrogen corp., Carlsbad, CA) by staining with ethidium bromide (0.25 $\mu\text{g}/\text{ml}$) and photographed under UV light. The

remaining PCR products were purified using the Qiagen QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol. The purified DNA fragments were sequenced in both directions at the UC Davis DBS Automated DNA Sequencing Facility. The chromatograms were processed with Chromas Lite v2.01 and visually inspected for correctness. The forward and backward sequences were visually reconciled using MEGA5 (Tamura et al. 2011, unpublished).

Phylogenetic analysis

We have downloaded and reviewed all *Cortinarius* ITS1/5.8s/ITS2 sequences available on GenBank (<http://www.ncbi.nlm.nih.gov>) and UNITE (<http://unite.ut.ee/>). During the initial analysis, we selected several hundred *Phlegmacium* sequences from the northern hemisphere based on the quality and representation of well-supported taxa. Added were approximately two hundred sequences from our *Phlegmacium* collections, mainly from California, but also from western North America and Europe. The phylogenetic analysis of that large dataset (not shown) clusters *Cortinarius xanthodryophilus* within the /pseudoglaucopodes/humolens clade.

A phylogenetic analysis of 19 sequences within the /pseudoglaucopodes/humolens clade (shown in FIG 1) contains 12 sequences from public databases and seven from our collections – three of *C. xanthodryophilus*, three collections of

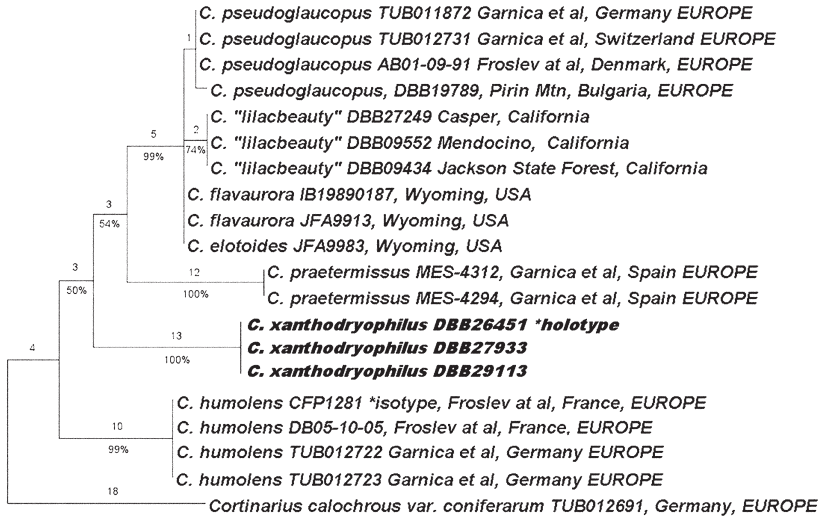


FIG 1. The single most parsimonious tree derived from partial nrDNA ITS sequence data showing the position of *Cortinarius xanthodryophilus* in relation to the other members of the Pseudoglaucopodes/Humolens clade. Branch lengths are shown above and the jackknife consensus numbers below the branches. The Bayesian inference (BI) analysis produced the same tree topology with very strong posterior probability support.

TABLE 1. List of the *Cortinarius* collections referenced in the phylogram (FIG 1).

TAXA	HOST TREE & LOCATION	VOUCHER NO.	GENBANK NO.
<i>C. calochrous</i> var. <i>coniferarum</i>	<i>Picea abies</i> , Hinterstein, Germany	TUB 012691	EU056956
<i>C. elotoides</i>	<i>P. engelmannii</i> , Wyoming, USA	JFA 9983	EU056948
<i>C. flavaurora</i>	<i>P. engelmannii</i> , Wyoming, USA	IB 19890187	AF325621
	<i>P. engelmannii</i> , Wyoming, USA	JFA 9913	EU056946
<i>C. xanthodryophilus</i>	<i>Quercus agrifolia</i> , California, USA	DBB26451 (*holotype)	HQ441244
	<i>Q. agrifolia</i> , California, USA	DBB29113	JF273637
	<i>Notholithocarpus densiflorus</i> , California, USA	DBB27933	JF273636
<i>C. humolens</i>	<i>Q. ilex</i> , Provence France	CFP1281	DQ663322
	<i>Fagus</i> sp., Provence, France	DB05-10-05	DQ663321
	<i>F. sylvatica</i> , Pfaffenweiler, Germany	TUB 012722	EU056955
	<i>F. sylvatica</i> , Ebringen (Schönberg), Germany	TUB 012723	EU056954
<i>C. "lilacbeauty"</i>	<i>P. sitchensis</i> , Casper, California, USA	DBB27249	JF273635
	<i>P. sitchensis</i> , Mendocino, California, USA	DBB09552	JF273634
	<i>P. sitchensis</i> , JSF, California, USA	DBB09434	HQ997909
<i>C. praetermissus</i>	<i>Q. ilex</i> subsp. <i>rotundifolia</i> , Spain	MES-4294	EU684534
	<i>Q. ilex</i> subsp. <i>rotundifolia</i> and <i>Pinus halepensis</i> , Spain	MES-4312	EU684535
<i>C. pseudoglaucopus</i>	Germany	TUB 011872	AY669573
	<i>P. abies</i> , La Chaux-de-Fonds, Switzerland	TUB 012731	EU056952
	Denmark	AB01-09-91	DQ663394
	<i>P. abies</i> , Pirin Mountain, Bulgaria	DBB 19789	JF273633

an undescribed species from California (provisionally labeled *C. "lilacbeauty"*), and one European collection of *C. pseudoglaucopus* (M.M. Moser) Quadr. from Bulgaria. *Cortinarius calochrous* var. *coniferarum* (M.M. Moser) Nezdobjm. was selected as the outgroup because it is fairly representative of the overall calochroid super-clade but falls outside of the /pseudoglaucopodes clade.

Multiple sequence alignments were generated with both ClustalX2 2.0.12 (Thompson 1997) and MAFFT v6.821b (Katoh 2002) with the G-INS-i global alignment iterative refinement strategy. The results were compared and visually inspected for areas of ambiguous alignment. The alignment and the molecular phylogenetic tree are available in TreeBase (<http://purl.org/phylo/treebase/phyloids/study/TB2:S11184>)

Maximum parsimony (MP) analysis was performed with PAUP* 4.0b10 (Swofford 2003) utilizing a heuristic search with tree-bisection-reconnection (TBR) branch swapping and 10000 random addition sequence replicates and maxtrees set at 1000. The analysis resulted in a single most parsimonious tree (FIG 1). To test branch length support, a Jackknife (JK) consensus tree from 1000 replicates was calculated with 50% majority rule.

In addition, Bayesian inference (BI) was run with Mr.Bayes v.3.1.1 (Huelsenbeck & Ronquist 2003) with the General Time Reversible substitution model plus gamma distribution (GTR + Γ) as the best fit recommended by MrModeltest v.2.3 (Posada & Crandall 1998). The BI ran two independent analyses with four chains for 1000000 generations with sampling frequency for every 100th generation and a burnin ratio set at 2500 (25%). The 50% majority rule consensus tree showed high posterior probabilities (PP) and produced the same topology as the most parsimonious tree (FIG 1).



FIG 2. *Cortinarius xanthodryophilus* (collection DBB26491) – the most typical form.

Taxonomy

Cortinarius xanthodryophilus Bojantchev & R.M. Davis, sp. nov.

FIGS 2–4

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Pileo 60–100 mm lato, hemispherico, dein plano-convexo, glutinoso, margine involuto, flavo, flavobrunneo, interdum rufo-brunneo maculato, *Lamellis emarginatis, pallide luteis, Stipite* 50–100 mm longo, *bulbo marginato* 30–50 mm lato, *Velo universale albido. Carne albida, cortina copiosa, rufo-brunnea, sapore miti. Sporis* 10–12 × 5.5–7 μm , amygdaliformibus usque limoniformibus, grosse verrucosis, *basidiis* 30–40 × 7–10 μm , tetrasporigeris, *fibulis praesentibus.*

TYPE: “USA, California, Contra Costa County, Tilden Park, Berkeley, 2009/11/15 col. Dimitar Bojantchev DBB26451 UCB Herbarium: **Holotype** UC 1860808 Genbank nrITS HQ441244”

ETYMOLOGY: from the Greek: *xanthos* = yellow, *drys* = oak, *philios* = loving

STATURE pileocarpous bulbopodium, very variable in aspect ratio. PILEUS 60–100 mm diam, hemispherical to convex to plano-convex to uplifted in age. Margin persistently involute. Colors rather uniform, predominantly in yellow



FIG 4. *Cortinarius xanthodryophilus*. a) DBB26451 (*holotype), b) DBB40412, c) DBB11176, d) DBB40412 showing KOH_{5%} reaction on pileus and context, e) DBB27933, f) DBB29113. All collections shown in FIG 4 have matching ITS1/5.8s/ITS2 sequences.

shades, starting pale straw- to sulphur-yellow (2.5Y 8/6) becoming yellow-brown (10YR 8/6-8/8), darker near the center (10YR 6/6-8/8), frequently with reddish-brown discolorations. Surface glutinous when wet, glabrous to dull glossy when dry, at age developing cracks and areolations near the disk, remaining smoother near the margin. LAMELLAE L=80–120, crowded, 8–15 mm broad, pale sulphur-yellow (2.5Y 8/6-8/8) to off-white when young, turning various shades of yellow-brown to brown (7.5R 6/6-5/6) as the spores mature. Edges even to occasionally slightly wavy, frequently eroded with age. Attachment notched. Lamellulae abundant, with widely varying extensions, 15–75%, series of 3–5. STIPE 50–100 mm long, 15–30 mm wide, cylindrical to subclavate above the bulb. Mostly white, but occasionally with light bluish tinges in the upper part. BULB 30–50 mm diam at the widest point, always well-developed, abruptly emarginated, tapering below, the subterrestrial part

with a white cottony mycelial felt. CONTEXT mostly white, slowly bruising brownish, few of the basidiomata feature a strong bluish cast in the upper stipe context, more pronounced near the surface. A watery grayish-blue cast above the lamellae is present in almost all basidiomata, rather faint in dry conditions, but persisting deep into maturity. UNIVERSAL VEIL white, frequently leaving floccose patches on the pileus surface, occasionally forming volva-like extensions on the bulb margin in recently expanded basidiomata. CORTINA white to pale yellow, turning rusty brown due to mature spore drop, copious, persistent, leaving an annular zone of dense fibrils on the stipe and frequently forming a hairy appendiculate zone on the pileal margin. The cortina deposits form a distinct brownish belt on the bulb edge, which can be fused into a gelatinized matrix on the periphery. MACROCHEMICAL REACTIONS KOH_{5%} light reddish brown on the pileus surface, stronger on the brownish spots near the disk. On the context the reaction varies from little to none on very young material, to yellowish-brown on mature basidiomata, stronger in the lower stipe and near the surfaces. No reaction was observed on the basal mycelium. UV no reaction was detected with both fresh and dry material. ODOR mild, leafy, and earthy. TASTE mild, earthy.

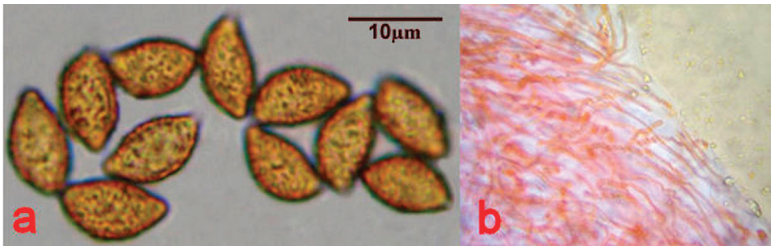


FIG 3. *Cortinarius xanthodryophilus*. a) Basidiospores b) Gelatinous cuticle.

BASIDIOSPORES (9.5–)10–12(–13) × (5–)5.5–7(–7.5) μm (mean 11.2 × 6.3 μm) Q = 1.66–1.85, Q_{av} = 1.78 (N = 213, 7 collections, 14 basidiomata), amygdaliform to citriform, distinctly and coarsely verrucose, deep rusty brown in deposit, slightly dextrinoid. BASIDIA 30–40 × 7–10 μm , 4-spored, cylindro-clavate, clamped. Hymenial layer not reacting to alkaline or iodine solutions. CYSTIDIA none observed. PILEIPELLIS a cutis, simplex, no hypodermium detected, composed of parallel to interwoven hyphae in a dense gelatinous matrix 150–250 μm thick. The outer 10–15 layers of hyphae 2–4 μm diam, entangled, some erect, irregularly shaped, strangulated to twisted, commonly with non-parallel walls, mostly with refractive cytoplasmic pigment. Lower layer of cuticle hyphae 3–7 μm diam, mostly parallel, with thicker yellow walls and hyaline content. The yellow pigmentation is emphasized when mounted in KOH. No distinct reactions to Melzer's reagent were observed. CLAMP

CONNECTIONS common in all parts. TRAMA composed of cylindrical cells 10–15(–20) μm diam, hyaline with pale yellow walls. Occasional oleiferous hyphae present.

HABITAT AND DISTRIBUTION – Solitary to gregarious under oaks. This species is common in California under live oak (*Quercus agrifolia*). In the Sierra Nevada foothills we have collected it under interior live oak (*Q. wislizenii*) and canyon live oak (*Q. chrysolepis*). In Mendocino Co. it was collected under tanoak (*Notholithocarpus densiflorus*). Based on a molecular data match there is a collection (Genbank DQ974721) under blue oak (*Q. douglasii*) in the Central Valley. There is also a collection (Genbank GQ159771) from Vancouver Island, British Columbia, under garry oak (*Q. garryana*).

ADDITIONAL COLLECTIONS EXAMINED: USA. CALIFORNIA: Contra Costa Co., Tilden Park, under *Quercus agrifolia*, 3 Dec 2008 (coll. D. Bojantchev DBB11492); 23 Nov 2009 (coll. D. Bojantchev DBB28181); 8 Dec 2008 (coll. D. Bojantchev DBB26491); San Mateo County, Huddart Park, under *Quercus agrifolia*, 8 Dec 2008 (coll. D. Bojantchev DBB11176, UCB Herbarium: UC 1860807); 8 Dec 2009 (coll. D. Bojantchev DBB29113); Marin County, Point Reyes, under *Quercus agrifolia*, 28 Nov 2006 (coll. D. Bojantchev DBB26128); San Mateo County, San Francisco Watershed, under *Quercus agrifolia*, 3 Dec 2010 (coll. D. Bojantchev DBB40412); Mendocino County, Casper Cemetery under *Notholithocarpus densiflorus* 22 Nov 2009 (coll. D. Bojantchev DBB27933).

Discussion

Phylogenetically, *C. xanthodryophilus* belongs to the /pseudoglaucopodes clade (Garnica et al. 2009) where it holds a well delineated position as shown in FIG 1. *Cortinarius xanthodryophilus* can easily be distinguished from species closely related to *C. pseudoglaucopus* (Jul. Schäff. ex M.M. Moser) Quadr. (FIG 5), which are typically associated with conifers and possess lilac veils (more or less obvious) and significantly larger spores than *C. xanthodryophilus*. *Cortinarius "lilacbeauty"* (FIG 6), the only clade member known to occur in California, is distinctly lilac-gray and thus easily separated in the field.

Amongst the hardwood-associated members of the clade, *C. xanthodryophilus* bears the closest resemblance to *C. humolens* Brandrud. Although they share many morphological features and the spores are very similar in size, shape and ornamentation, only *C. xanthodryophilus* has frequent bluish tinges on the context and upper stipe. For a good treatise and iconography of *C. humolens*, refer to Brandrud et al. (1998), Bidaud et al. (2004), and Consiglio et al. (2007).

Another closely related species, *C. praetermissus* Bergeron ex Reumaux, was collected from Morella, Eastern Spain, under evergreen oaks. *Cortinarius praetermissus* is not well known, but it was originally described as a beech-associated, pale bluish species. Its spore shape and size, which fit quite well with the species close to *C. pseudoglaucopus*, are larger than those of *C. xanthodryophilus*.



FIG 5. *Cortinarius pseudoglaucopus* collections DBB19789 and DBB19822 from the Pirin Mountain Bulgaria, Europe, under *Picea abies*, showing the full range of coloration in younger and older basidiomata.



FIG 6. *Cortinarius* "lilacbeauty" collections. DBB 09434 and DBB 09552 from Northern California, under *Picea sitchensis*. Based on the partial nrITS sequences this species is very close to *Cortinarius pseudoglaucopus* (FIG 5).

Other yellow bulbopodiums in the northern California mixed woods add to the challenge of identifying *C. xanthodryophilus* in the field. The local species most likely to be confused with *C. xanthodryophilus* is "*Cortinarius fulmineus*" sensu Moser & Ammirati 1997 (FIG 7). This species, which shares the same general colors with *C. xanthodryophilus*, tends to oxidize red-brown on the pileus, particularly on the disk. It also has a strong alkaline reaction, which is purple red on the pileal surface and pinkish on the context, as well as smaller spores (8–10 μm). *Cortinarius elegantior* var. *americanus* M.M. Moser & McKnight (FIG 8), a conifer-associate that can be confused with *C. xanthodryophilus* and frequently occurs with it in mixed woods, can be differentiated by the strong red alkaline reaction on the pileus and bulb and the much larger spores.

There are several other common and undescribed yellow bulbopodiums in Northern California that can be confused with *C. xanthodryophilus*. Genetically, they fall in the clades around *C. citrinus* P.D. Orton, *C. elegantissimus* Rob. Henry, *C. flavovirens* Rob. Henry, *C. fulvocitrinus* Brandrud, *C. platypus*



FIG 7. "*Cortinarius fulmineus*" sensu Moser & Ammirati 1997. This species is the most likely to be confused with *C. xanthodryophilus* in the field. The pronounced tendency of the pileus of even young basidiomata to oxidize red-brown and the strong $\text{KOH}_{5\%}$ reaction (lower right photo) aids field identification.



FIG 8. *Cortinarius elegantior* var. *americanus*, col. DBB27264.
 $\text{KOH}_{5\%}$ reaction on the right.

(M.M. Moser) M.M. Moser, *C. xanthophyllus* (Cooke) Rob. Henry, and others. A complete treatment of these species is beyond the scope of this article, but they all differ from *C. xanthodryophilus* by the combination of macromorphology, macrochemical reactions, and microscopic detail. In a future publication we will provide a comprehensive key to the *Phlegmacium* species of Northern California with an emphasis on field level identification.

A complete iconography of *Cortinarius xanthodryophilus* and a comparative image study is available on the website <http://www.mushroomhobby.com>.

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