

A new *Cortinarius* of section *Calochroi* (Basidiomycota, Agaricomycetes) from Mediterranean *Quercus* woodlands (Italy)

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Abstract: A new species of *Cortinarius*, *C. flavoaurantians* sp. nov., is described from Italian *Quercus* woods based on both morphological and ITS rDNA data. This taxon is characterized by a yellowish pileus and cortina, a white universal veil and a pileipellis that reacts yellow-orange with KOH. Illustrations of the key micromorphological features and fresh basidiomata in situ are provided. Closely related species are discussed.

Key words: Agaricales, biodiversity, Cortinariaceae, *Cortinarius calochrous*, taxonomy

INTRODUCTION

The /calochroid clade is a species-rich lineage of *Cortinarius* (Pers.) Gray (Cortinariaceae R. Heim ex Pouzar, Agaricales Underw.), mainly distributed across the forested northern hemisphere and formerly classified in subgenus *Phlegmacium* (Fr.) Trog (now regarded as an artificial polyphyletic group; Garnica et al. 2003, 2005, 2009; Peintner et al. 2001, 2004; Frøslev et al. 2005; Harrower et al. 2011). It comprises no less than 100 species occurring throughout Europe, Central and North America (Frøslev et al. 2007; Garnica et al. 2009, 2011; Harrower et al. 2011), characterized by conspicuous, brightly pigmented basidiomes, a dry stipe with a rounded to abruptly bulbous (marginate) base, a pileipellis with a well developed gelatinous matrix, and the presence of pigments (in pileus and stipe surface and context), which often change color with the application of KOH.

This clade includes sections *Calochroi* M.M. Moser & Horak (“calochroid” taxa sensu Frøslev et al. 2007, without anthraquinoid pigments) and *Fulvi* M.M. Moser (“fulvoid taxa” sensu Frøslev et al. 2007, with anthraquinoid pigments) as traditionally circumscribed by Melot (1990), Brandrud (1998) and Brandrud et al. (1989–1998), and is regarded as one of the most difficult taxonomic groups in the Agaricales (Frøslev et al. 2007).

Recently seven major monophyletic lineages were recognized by Garnica et al. (2009) within the /calochroid clade based on a combined multilocus phylogenetic analysis, namely *Calochroi* (hereafter indicated as *Calochroi* s.s.), *Caroviolacei*, *Dibaphi*, *Elegantiores*, *Napi*, *Pseudoglaucopodes* and *Splendentes*. Within *Calochroi* s.s. several species are rare and have limited distributions, often growing on calcareous soils and mainly associated with deciduous trees belonging to Betulaceae (*Corylus*, *Carpinus*, *Ostrya*) and Fagaceae (*Castanea*, *Fagus*, *Quercus*); a few species are known to be associated with Pinaceae (Garnica et al. 2009). Most species are characterized by bright (pink-red) reactions of the basidiome surface to KOH, usually indicating the presence of sodagnitins (Sontag et al. 1999; Frøslev et al. 2007), lilac lamellae, presence of abundant and evident universal veil remnants on pileus and stipe, absence of anthraquinoid pigments (Gill and Steglich 1987; Frøslev et al. 2007), and an eight bp length indel in the intron 2 portion of the RPB1 gene (Garnica et al. 2009).

To date, species circumscriptions have relied on morphological characters, but a confident specific assignment can hardly be made on the basis of morphological data alone, even if both micro- and macroscopic characters are taken into account. Variability and/or overlapping of many important characters, such as basidiome colors, spore dimensions, host-tree specificity, and even chemical reactions with KOH, all lead to problematic and conflicting taxonomic treatments. Recently analyses of genomic sequences (ITS, RPB1-RPB2 and D1/D2 regions of the nucLSU rDNA) have provided considerable aid (Frøslev et al. 2006, 2007; Ortega et al. 2008; Garnica et al. 2009). The number of species recognized to date based on phylogenetic analyses of gene sequences (Frøslev et al. 2006, 2007; Garnica et al. 2009) has proved to be comparable or larger than that previously proposed even by authors such as Bidaud et al. (2001) who employ a narrow

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morphological species concept. However species recognized by means of a morphological species concept (morphospecies) and those recognized by applying a phylogenetic species concept (phylopecies) often do not overlap and in many cases a single name was applied to different phylogenetic species, or vice versa different names were given to the same phylogenetic species. It is evident that a correct, phylogenetic taxonomic framework can be achieved only by using both morphological and molecular markers; in *Calochroi* this goal is still far from reach.

During fieldwork in *Quercus* woodlands in Liguria (NW Italy), collections of a striking *Cortinarius* of *Calochroi* s.s with a white universal veil, yellowish cortina, and a peculiar yellow-orange reaction of the pileus surface to KOH, were made. Based on both morphological and ITS rDNA analyses, the taxon is described as a new species.

MATERIALS AND METHODS

Morphology.—Macromorphological features were described from fresh specimens. Microscopic elements were described from herbarium material rehydrated in 3% KOH and stained in Congo red. The localization of the pigments in the pileipellis elements was observed from water mounts of dried material. Basidiospore measurements are based on means of 120 spores in 3% KOH from three collections. Only mature, normally developed and non-aberrant spores from spore prints (e.g. from the cortina remnants on the stipe) were measured. The width of each basidium was measured at the widest part, and the length was measured from the apex (sterigmata excluded) to the basal septum. These abbreviations are used: L = number of entire lamellae; l = number of lamellulae between each pair of entire lamellae; Q = the quotient of length and width of the spores in side view; Q_m = average quotient. Alkaline reactions were produced by adding a drop of potassium hydroxide (10% KOH) to surfaces (pileipellis, stipitipellis, bulbipellis) and context (KOH drop test), and observed on young as well as on mature and dried specimens. Color terms in capital letters (e.g. Pale Orange-Yellow) are those of Ridgway (1912). Author citations follow the Index Fungorum Authors of Fungal Names (<http://www.indexfungorum.org/authorsoffungalnames.htm>). Herbarium abbreviations are according to Thiers (2011). The type material is kept in GDOR (Herbarium of the Museo Civico di Storia Naturale Giacomo Doria, Genoa, Italy).

DNA extraction, PCR amplification and DNA sequencing.—Genomic DNA was isolated from 1 mg of a dried herbarium specimen from three collections (GDOR 2528, GDOR 2529, GDOR 2530) with the DNeasy Plant Mini Kit (QIAGEN, Milan, Italy) according to the manufacturer's instructions. Universal primers ITS1F/ITS4 were used for the ITS region amplification (White et al. 1990; Gardes and Bruns 1993). Amplification reactions were performed in a PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) following Vizzini

et al. (2011). PCR products were purified with the AMPure XP kit (Beckman) and sequenced by MACROGEN Inc. (Seoul, Republic of Korea). The sequences were assembled and edited with the phred/phrap/consed software suite. Sequences were submitted to GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>), and accession numbers are reported in *Material studied* and in FIGS. 1, 2; the alignments and phylogenetic trees are available at TreeBASE (www.treebase.org) under accession number 12646.

Sequence alignment and phylogenetic analysis.—The sequences were checked and assembled with Geneious 5.3 (Drummond et al. 2010) and compared to those available in GenBank database with the BLASTn algorithm. Based on the BLASTn results, *Cortinarius* sequences were selected according to the outcomes of recent phylogenetic studies on *Cortinarius* sect. *Calochroi* (Frøslev et al. 2007; Ortega et al. 2008; Garnica et al. 2009). Alignments were generated with MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The alignment was imported into MEGA 5.0 (Tamura et al. 2011) for manual adjustment. The best-fit models were estimated by both the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with jModelTest 0.1.1 (Posada 2008) to provide a substitution model for the alignment. The GTR + G substitution model was chosen for the analyses. Phylogenetic analyses were performed with maximum likelihood (ML) and Bayesian inference (BI) approaches. Three *Cortinarius* species belonging to sect. *Elegantiores* (*C. elegantior* [AY174850], *C. eufulmineus* [EF014257], *C. majusculus* [EU655682]) were used as outgroup taxa in both analyses. ML estimation was performed through RAxML 7.0.4 (Stamatakis 2006) with 1000 bootstrap replicates (Felsenstein 1985) with the GTRGAMMA algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree with the -FA option of RAxML and -x 12345 as a random seed to invoke the novel rapid bootstrapping algorithm. BI of phylogeny with Monte Carlo Markov chains (MCMC) was carried out with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Four incrementally heated simultaneous MCMC were run 10 000 000 generations. Trees were sampled every 1000 generations, resulting in an overall sampling of 10 001 trees. The first 2500 trees (25%) were discarded as burn-in. Burn-in value was determined with Tracer 1.5 (Rambaut and Drummond 2007). For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPP). Branch lengths were estimated as mean values over the sampled trees. Only MLB more than 50% and BPP values more than 0.70 are reported in the resulting trees (FIGS. 1, 2). Pairwise percent identity values of ITS sequences were calculated with MEGA 5.0 (Tamura et al. 2011).

RESULTS

Phylogenetic analyses.—The amplification of the ITS regions was successful for the three specimens,

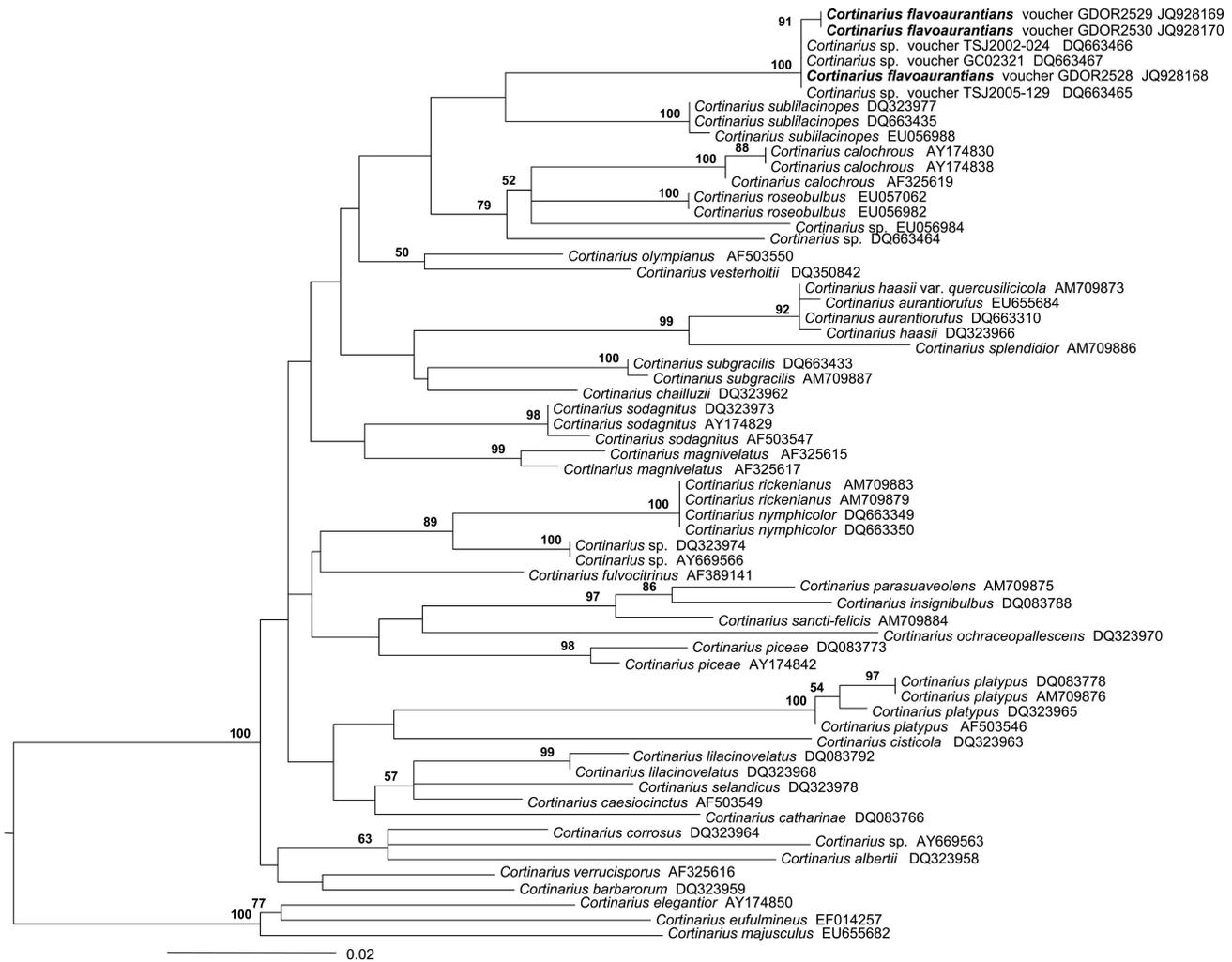


FIG. 1. Maximum likelihood phylogram obtained from the ITS (ITS1-5.8S-ITS2) sequence alignment of *Cortinarius* spp. (sect. *Calochroi* s.s.). *C. elegantior*, *C. efulmineus* and *C. majusculus* (sect. *Elegantiores*) were used as outgroup taxa. MLB values greater than 50% are above branches. Bar = number of substitutions per site.

yielding a PCR product of about 680 bp. The ITS data matrix comprised a total 62 sequences (including 59 from GenBank). This dataset is 646 bp long and contains 197 (30.5%) variable sites. Of these, 128 (19.8%) are parsimony informative.

In both ML and Bayesian analyses, our three *Cortinarius flavoaurantians* sequences cluster with three sequences belonging to unidentified *Cortinarius* collections (GC02321, TSJ2002-024, TSJ2005-129), forming a well supported clade (MLB 100% and BPP 1; FIGS. 1, 2). These three unidentified collections correspond to the *Cortinarius* sp. 7 in Frøslev et al. (2007). The pairwise percent identity value of the entire clade is 99.9. The ML analysis placed these six sequences in a weakly supported sister clade to *C. sublilacinopes* (MLB <50%), while in the BI tree they are sister to a clade consisting of *C. calochrous*, *C.*

roseobulbus and two *Cortinarius* sp. (<0.70 BPP). In the BI tree, the clade composed of *C. flavoaurantians*, *C. calochrous*, *C. roseobulbus*, *C. sublilacinopes* and five *Cortinarius* sp. is supported by 0.99 of BPP.

TAXONOMY

Cortinarius flavoaurantians Boccardo, Clericuzio & Vizzini, sp. nov. FIGS. 3–5
Mycobank MB800140

It is characterized by an initial yellow-orange KOH reaction of pileus surface (becoming red-brown in a few minutes) in fresh basidiomata, bulbipellis and stiptipellis not reacting with KOH, white universal veil remnants, a yellowish partial veil, white mycelial strands and small amygdaliform spores (<10 µm on average).

Type: Italy, Liguria, Genoa, M. Portofino, 19/11/2011, leg. F. Boccardo, (GDOR 2529, Holotype).

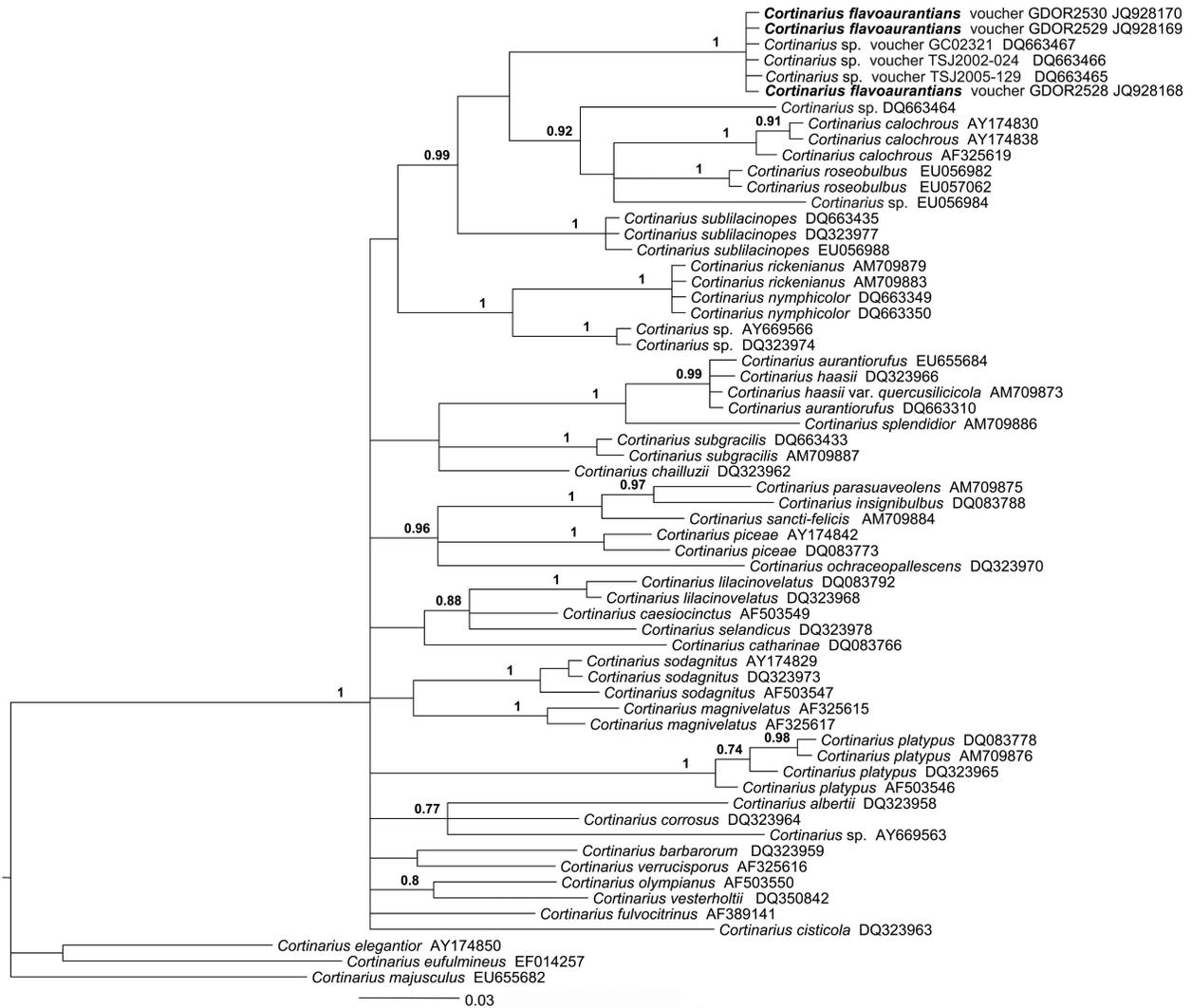


FIG. 2. Bayesian phylogram obtained from the ITS (ITS1-5.8S-ITS2) sequence alignment of *Cortinarius* spp. (sect. *Calochroi* s.s.). *C. elegantior*, *C. efulmineus* and *C. majusculus* (sect. *Elegantiores*) were used as outgroup taxa. BPP values greater than 0.70 are given above branches. Bar = number of substitutions per site.

Pileus 30–60 mm broad, initially convex, then plano-convex with an involute margin, finally slightly depressed at center with age; surface viscid, at first smooth, sometimes slightly wrinkled, light pale yellow (Pale Orange-Yellow, Maize Yellow), to ochraceous yellow (Buff-Yellow), soon spotted with reddish brown to rusty orange (Deep Chrome, Cadmium Yellow) \pm radial innate fibrils; universal veil remnants abundant on pileus surface, at first as white, large thin patches, sometimes with a few fugacious violaceous hues (Pale-Blue Violet) (FIGS. 3b, 4), then bruising orange-brown (Orange Rufous, Xanthine Orange, Mars Orange) and visible as small scales. Lamellae emarginate, medium crowded (L = 60–80, l = [0] 1–2 [3]), violaceous pink to light purplish at the beginning (Pallid Violet-Blue, Pallid Blue-Violet,

Pallid Bluish Violet, Pallid Violet), rapidly darkening to rusty brown with age (due to spore maturation); with a slightly crenulate edge. Stipe 20–70 \times 10–20 mm, stout, whitish to pale yellowish, usually showing pink-violet tinges (Pallid Violet-Blue, Pallid Blue-Violet) at apex, cylindrical, with a broad, flattened marginate bulb (\times 20–35 mm); universal veil remnants abundant on bulb surface, white, sometimes with a few fugacious scattered violet hues (Pale-Blue Violet); partial veil (cortina) abundant, yellowish; mycelial strands white. Context white, slightly pink-violaceous at stipe apex; odor weak, somewhat herbaceous; flavor mild but bitter in the pileus surface. Chemical reactions 10% KOH on pileus surface of fresh basidiomes, at first dark yellow (Apricot yellow, Cadmium Yellow), in few seconds



FIG. 3. *Cortinarius flavoaurantians*. a. Basidiomes (from GDOR 2529, holotype). b. Basidiomes with universal veil remnants with fugacious violaceous hues (from GDOR 2528). Color spots on pileus surface are due to KOH application (10% KOH drop test). Bars = 20 mm.

orange-yellow, carrot orange (Cadmium Orange, Ochraceous Orange, Carmelian Red), then quickly oxidizing to red or reddish brown (Xanthine Orange, Tawny, Ferruginous) (FIG. 3a, b); weak or no reaction on bulbipellis, stipitipellis and context; 10% KOH on pileus surface of dried basidiomes, red to reddish brown (Xanthine Orange, Tawny, Ferruginous).

Spores $(8.2)8.5\text{--}10.5(11.0) \times 5.3\text{--}6.3(6.7) \mu\text{m}$, $9.31 \times 5.89 \mu\text{m}$ on average, $Q = (1.36)1.42\text{--}1.79(1.84)$, $Q_m = 1.58$, regularly amygdaliform, more rarely elliptic-amygdaloid, never or rarely papillate-citri-form, distinctly and coarsely verrucose (FIG. 5c, d); warts large, up to $1.5 \mu\text{m}$ high, mainly isolated or slightly connected. Basidia clavate, four-spored, $30\text{--}35 \times 8\text{--}9 \mu\text{m}$, thin-walled and hyaline in KOH; sterigmata up to $5\text{--}6 \mu\text{m}$ long; basidioles $20\text{--}30 \times 6\text{--}8 \mu\text{m}$ (FIG. 5b). Cheilocystidia and pleurocystidia not observed. Pileipellis (FIG. 5a, e) an ixocutis with some repent and ascending hyphae, made up of long, broad cylindraceous to fusoid elements, typically with distant septa; terminal elements $50\text{--}100(140) \times 4\text{--}$



FIG. 4. *Cortinarius flavoaurantians*. Basidiomes (mixed specimens from GDOR 2528, GDOR 2529 and GDOR 2530). Drawing by F. Boccardo. Bar = 20 mm.

$6(7) \mu\text{m}$ with an obtuse to subcapitate apex, sometimes nipple-shaped (FIG. 5e); subpellis of interwoven, somehow short and broad hyphae, for example $35\text{--}60 \times 5\text{--}10(15) \mu\text{m}$. Pigment in outer hyphae mainly parietal, distributed as a thick golden yellow coating, often also more or less finely incrusting; in deeper hyphae parietal and coarsely encrusting; a more scattered cytoplasmic yellow pigment is found as granules in some outer hyphae. Clamp connections frequent at all septa.

Etymology: The epithet, derived from the Latin words *flavus* (yellow) and *aurantians* (becoming orange), refers to the pileus and to the yellow-orange reaction of the pileus surface to KOH.

Habitat: Mid- to late autumn (October, November), under broadleaf trees, mainly *Quercus pubescens* and *Ostrya carpinifolia*, on calcareous soil. Often growing thickly gregarious to (sub)caespitose.

Material studied: ITALY, Liguria, Genoa, Zoagli, about 300 m a.s.l., under *O. carpinifolia*, *Q. pubescens* and scattered *Q. ilex*, leg. F. Boccardo, 17 Nov 2011 (GDOR 2528, GenBank JQ928168); Genoa, M. Portofino, north-eastern slope, about 500 m a.s.l., under *Q. pubescens* and *O. carpinifolia*, leg. F. Boccardo, 19 Nov 2011 (GDOR 2529,

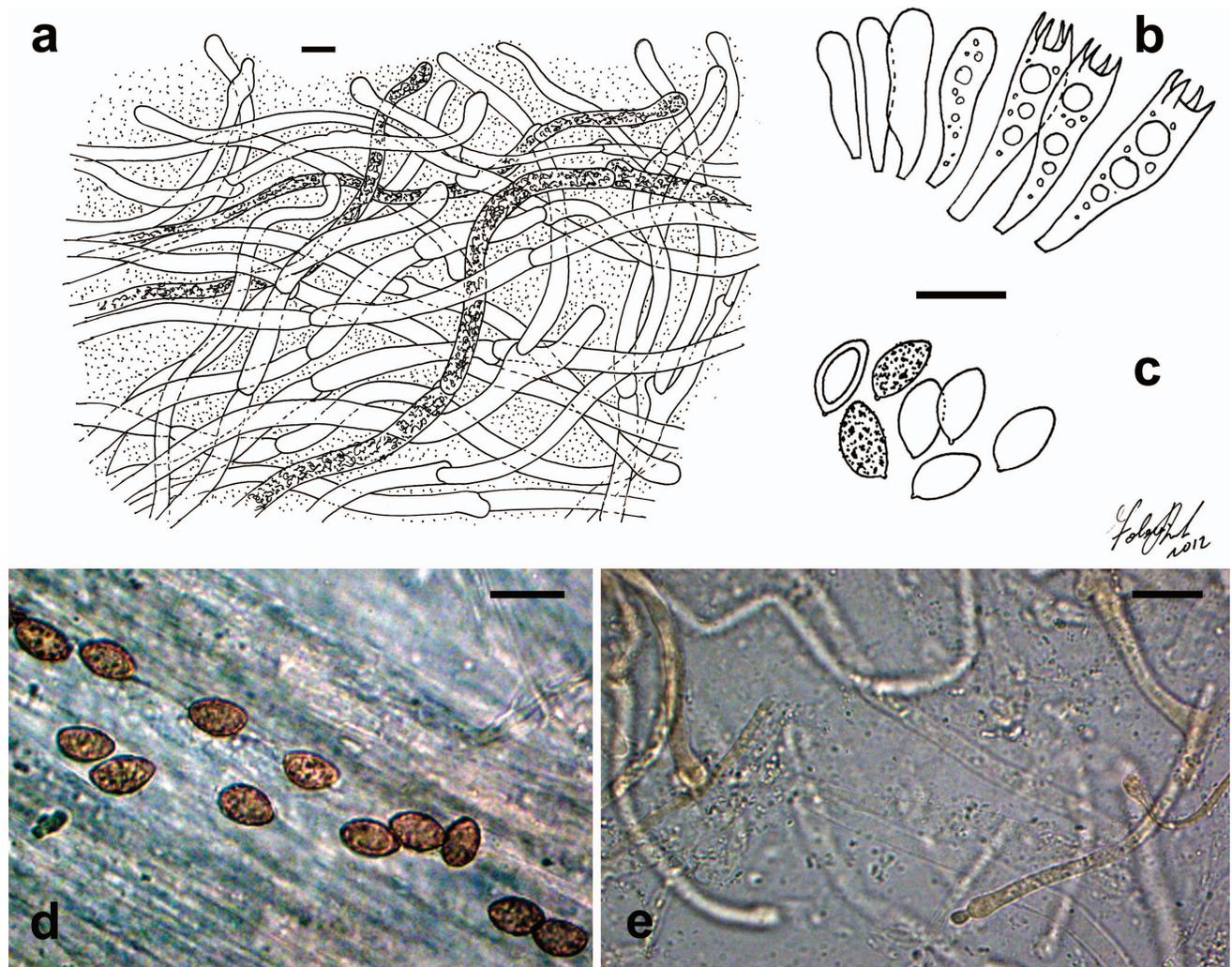


FIG. 5. *Cortinarius flavoaurantians*. Microscopic features (from GDOR 2529, holotype). a, e. Elements of the pileipellis. b. Basidia and basidioles. c, d. Spores. Bars = 10 μ m.

GenBank JQ928169, HOLOTYPE); ibidem, but at about 400 m from the first collection site, leg. F. Boccardo, 23 Nov 2011 (GDOR 2530, GenBank JQ928170).

DISCUSSION

Morphological data and phylogenetic analyses of ITS sequences support recognition of our collections as an independent species within *Calochroi* s.s., *C. flavoaurantians*.

As noted above, three unnamed collections (GC02321, Italy, Emilia-Romagna, under *Q. pubescens* and *Q. cerris*; TSJ2002-024, Czech Republic, under *Carpinus* and *Quercus*; TSJ2005-129, Spain, under *Q. ilex* and *Q. pubescens*), corresponding to *Cortinarius* sp. 7 in Frøslev et al. (2007), show a complete ITS sequence homology with *C. flavoaurantians* (pairwise percent identity value = 99.9). Sequences from these three undetermined collections were considered

close to either *C. sublilacinopes* and *C. calochrous* (Frøslev et al. 2007) or to *C. calochrous* and *C. roseobulbus* (Garnica et al. 2009). Future molecular analyses including additional gene sequences for increased resolution could provide additional evidence for considering these collections conspecific to *C. flavoaurantians*; at the moment data are insufficient to draw this conclusion.

Morphologically *C. flavoaurantians* belongs to a complex of species within *Calochroi* s.s. whose pilei are predominantly shades of yellow (pale yellow to ochraceous yellow, yellow-brown), and so finds its traditional placement close to species such as *C. albovestitus* Bidaud, *C. calochrous* (Pers.) Gray, *C. catharinae* Consiglio, *C. chailluzii* Frøslev & T.S. Jeppesen, *C. cisticola* Frøslev & T.S. Jeppesen, *C. frondosophilus* Bidaud, *C. lilacinovelatus* Reumaux & Ramm, *C. roseobulbus* M.M. Moser, *C. sublilacinopes* Bidaud, Moënne-Locc. & Reumaux, and *C. subgracilis*

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