Cortinarius mahiquesii, a new subhypogeous species from Catalonia (Iberian Peninsula)

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Key words
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INTRODUCTION

Cortinarius belongs, together with Entoloma, to the larger agaric genera (Noordeloos 2004, Peintner et al. 2004). Although these genera are fairly well known, numerous new species are still to be expected, not only from extra-European areas (e.g. Peintner et al. 2003, Manimohan et al. 2006, Gates & Noordeloos 2007), but also from Europe. In recent years, numerous papers, including both evolutionary and taxonomic studies, have been published about the genus Cortinarius (subgenus Phlegmacium) in Europe (Garnica et al. 2003, 2005, Freslev et al. 2005, 2006, 2007, Bidaud et al. 2008, Ortega et al. 2008). In spite of this extensive research into European Cortinarius, the description of new species is quite frequent, especially in certain Mediterranean ecosystems that were poorly studied in the past. This is the case of the Cistus scrub communities in north-eastern Spain (Catalonia), where recent studies have shown their very particular mycobiota (especially mycorrhizal macrofungi) and have led to the description of several new taxa (Vila & Llimona 2002, 2006; Ballarà et al. in press); C. aureocistophilus, C. limonae, C. xanthosarx, etc.

In this paper we propose a new species, Cortinarius mahiquesii, collected in the Alt Empordà (Girona) in a pure Cistus monspeliensis scrub community (NE of the Iberian Peninsula). Drawings of microscopic characters, scanning microphotographs of the basidiospores, and colour pictures of the basidiomes in their natural habitat are provided. A molecular analysis was carried out to characterize the new species using ITS sequences.

MATERIAL AND METHODS

The macromorphological study was carried out on both young and mature fresh specimens. For the macrochemical test, we used KOH 15–40 % solution on cap, bulb and context. In order to define the basidiome habit, we used the following parameters: slenderness index (Is = D/Dx); I = stipe length, D = pileus diam, d = stipe width; according to Kuyper 1986; pileus diam/stipe length (Pd/Si); stipe length/pileus width (Si/Sw) and stipe width/bulb width (Sw/Bw). The micromorphological analysis was performed using free-hand radial sections of pileus and longitudinal sections of the gills mounted in KOH (3 %) and Congo red (in NH₄OH). Twenty spore measurements of several basidiomes were made and the minimum, mean and maximum values, as well as the length/width ratio (Q = L/w) were calculated. The spore ornamentation was recorded using a Field Emission Scanning Electron Microscope Leo (Zeiss), model 1539 Geminis (FESEM). The material studied is housed in the herbaria GDA (University of Granada, Spain) and JVG (personal herbarium of the first author). The classification of Cortinarius follows Brandrud et al. (1994), Freslev et al. (2005), Garnica et al. (2005), Consiglio et al. (2007b), and Bidaud et al. (2008).

For the molecular analysis, the total genomic DNA was extracted using the CTAB method (Doyle & Doyle 1987). The entire ITS region (ITS-1, 5.8S and ITS-2) was amplified by PCR, using primers ITS-5 (White et al. 1990) and C26A (Wen & Zimmer 1996). The PCR reactions were performed in a volume of 50 µl under standard conditions (Innis et al. 1990). The ITS region was amplified for C. mahiquesii and its most closely related species (taken from GenBank). The selection of sequences taken from the GenBank was based on the following: the morphological analyses carried out by us; the results of Garnica et al. (2003, 2005) and Freslev et al. (2005) and BLAST searches. The EMBL accession number for C. mahiquesii is included in the Specimens examined section, and the GenBank accession number for the remaining sequences are shown in the ITS tree. The ITS sequences were aligned using the CLUSTAL option of the MEGALIN program from the DNA-
star software package (LASERGEN), followed by a visual inspection. The genetic distances (uncorrected $p$-distance) between the sequences were calculated using MEGA version 4 (Tamura et al. 2007).

Phylogenetic analysis was performed using maximum likelihood (ML) criterion, as implemented in PAUP* 4.0b10 (Swofford 2003). The best-fit model of nucleotide substitution for the ITS data was Felsenstein 81 (Felsenstein 1981) with variable sites following a discrete gamma distribution (F81 + G), as implemented in Modeltest 3.06 (Posada & Crandall 1998). ML searches were implemented with a starting tree obtained via Neighbor Joining (NJ) and the Jukes-Cantor model, and using tree bisection-reconnection (TBR) branch-swapping. Gaps were treated as missing data. Bootstrap values were calculated to assess branch support in the resulting trees. These analyses were performed using 1 000 pseudoreplicates and TBR branch swapping beginning with the topology resulting from the NJ analysis. Fig. 1 shows the maximum likelihood tree showing the relationships between C. mahiquesii and its most closely related species.

**Taxonomy**

*Cortinarius mahiquesii* Vila, A. Ortega & Suár.-Sant., sp. nov.
— MycoBank MB512434; Fig. 2–4

Basidiomata subhypogea. Pileus usque 40 mm in diam, convexo-applanatus, griseus vel griseo-azureus, saepe leviter olivaceus, per aetatem luteo-maculatus; superficies viscida, deinde sicca, ut videtur leviter velutina. Lamellae primo azureae, per maturitatem brunneo-griseae. Stipes usque 30 × 11 mm, bulbo debiliter marginato, prominenti aut mediocri, munitus; primo azureus, deinde albescens coloratus; siccus; annullaria zona bene delimitata; per aetatem lutescens. Caro a farina odorus. Sporae 9.8–12 × 5–6.1(−6.4) μm, amygdaloideae, suboliviformes vel oviformes (Q: L/w = 1.86–2.1), leviter usque aliquantum valde verrucoseae, dense conexus tuberculis obtectae. Acierum sterilium cellulae abundantes, 20–25 × 6.5–9.5 μm, cylindraceae, clavatae, sub-oliviformes vel subpyriformes. Pileipellis duplex; epicutis a numerosis, septatis, crassitunicatis, 80–180 μm longis, apicalibus elementibus composita. — Holotypus (hic designatus) prope urbem el Port de la Selva dictam, in Catalonia (Hispania), a J. Vila et X. Llimona lectus, 18-1-2008, 240 m altitudinis, sub *Cistus monspeliensis*, in herb. GDA 54298 conservatus.

*Etymology.* This species is dedicated to Rafael Mahiques Santandreu (Quatretonda), due to his significant contribution to the study of the genus *Cortinarius* in the Iberian Peninsula.

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**Fig 1** Maximum likelihood tree (-lnL = 2099.12) showing the relationships between *Cortinarius mahiquesii* and its most closely related species. Bootstrap values ≥ 50 % are shown above branches. The accession number (for the sequences taken from GenBank) and/or herbarium references are shown before and after each taxon name respectively.
Basidiomes subhypogeous, isolate, gregarious or forming small clusters, stocky habit (Is = 1.13–1.44–1.78). Pileus up to 40 mm in diam, convex or hemispheric, later convex-flattened, rarely slightly depressed towards the centre; firstly bluish, mainly at the margin and in specimens that are still buried, later greyish, bluish grey or brown-grey, with olivaceous hues, which intensify in mature basidiomes; in older specimens, the pileus is intensely yellowing or brown-yellow all over. The surface is glutinous, mainly when young, although it soon dries out; typically, it has abundant adhered earth particles, as the pileus surface acquires a fibrous-downy or fibrous-velvety look during the fruiting process, mainly in the disc, and hardy fibrous towards the margin. Margin involute, partially straight in mature specimens, slightly undulated in adult basidiomes. Lamellae adnate or sinuate, dense, rather thin, sometimes bifurcate, firstly bluish, later becoming bluish grey, grey-beige and, finally, grey-brown; edge paler and slightly irregular; lamellulae abundant (1 : 1 to 1 : 3). Spore print ferruginous. Stipe up to 30 × 11 mm, shorter than the pileus diameter (Pd/Si = 1.43–1.53–1.68), stocky to moderately slender (Pl/Pw = 1.4–2.02–2.67) with a poorly marginate, prominent to moderate bulb (Sw/Bw = 0.52–0.62–0.68) up to 17 mm in diam; bluish when young, rather intense later, when maturing, whitish or slightly greyish; surface dry, with thin fibrilles at the apex and a typical, well-delimited annular zone, which forms a faint circular line around the stipe; with whitish mycelial strands; when specimens become older, the stipe intensely yellowing to yellow-brown, like the pileus. Context rather compact, whitish or creamy, with bluish hues at the apical part of the stipe and bluish grey under the pileipellis and above the lamellae, yellowing in the basal part of the bulb; a slightly floury smell, becoming partially raphanoid in older specimens. KOH reaction yellowish, darkening on the context, and dark brown-violaceous on the pileipellis.
Spores 9.8–12 × 5–6.1(–6.4) μm, mv: 10.7–11.2 × 5.4–5.8 μm, rather strongly dextrinoid (following Vesterholt 2005), amygdaloid, subcitriform to citriform (Q: L/w = 1.86–2.1; mv: 1.93–1.96), moderately to rather strongly verrucose with ± densely interconnected warts. Basidia 25–35 × 7.5–10 μm, 4-spored, cylindrical to clavate, forming a ± dense palisade. Lamellar edge sterile cells abundant, 20–25 × 6.5–9.5 μm, cylindrical, clavate, subutrifor or subpyriform. Subhymenium differentiated, ramose, made up of jumbled (‘puzzle’) little cells (c. 1.5 μm wide). Hymenophore trama ± parallel, formed by pale, 5–10 μm wide hyphae. Pileipellis duplex. Epicutis structure a scarcely gelified ixocutis, with tendency to become a trichoderm, formed by flexuous and straight terminal elements, moderately to strongly thick-walled, septate, with a granular greyish pigment, 80–180 μm long, sometimes with enlarged basal cells. The apical elements show a variable shape (cylindrical, claviform or subcapitate), 5–10 μm wide. Hypocutis differentiated, with ± parallel or slightly interwoven, up to 10–22 μm wide hyphae with numerous septa, which delimit short cellular elements, disposed in a pseudo-parenchymatous (subcellular) structure.

Specimens examined. SPAIN. Catalonia. Perafita, el Port de la Selva (Girona), alt. 240 m, subhypogeous, under Cistus monspeliensis, on acid soil, 18 January 2008, leg. J. Vila & X. Llimona, GDA 54298 (holotypos, designated here) (EMBL accession number FM202139), isotypus in JVG 1080118-18.

Taxonomic notes

On the basis of its morphological features, C. mahiquesii belongs to sect. Glaucopodes, subsect. Amoenolentes (Bidaud et al. 2008), or, according to Consiglio et al. (2007a), is part of subsect. Magici. From a molecular point of view, C. mahiquesii relates to the Glaucopodes (Garnica et al. 2003, Frøslev et al. 2005) or Caerulescentes (Garnica et al. 2005) clades, and relates to the Glaucopodes (Garnica et al. 2003, Frøslev et al. 2008), or, according to Consiglio et al. (2007a), is part of Amoenolentes (Cortinarius anserinus (= C. amoenolens) and C. viridocoeruleus. The separation of these species from C. mahiquesii can clearly be seen in the basidiomate morphology and the lack of a farinaceous smell. Cortinarius anserinus is distinguished by its: i) epigeous growth; ii) different habitat (nemoral broad-leaved forests); iii) faint, pleasant-sweetish, not farinaceous smell; and iv) bitter taste of the cap cuticle. With regards to the second species, mycologists (Bidaud et al. 2008) currently distinguish two ecological variants: a) C. viridocoeruleus var. camptoros growing in coniferous forests; and b) C. viridocoeruleus var. viridocoeruleus, fruiting in the warm Quercus forests and more prevalent in the Iberian Peninsula than the former variety (Mahiques 2006). Consiglio et al. (2007a) also distinguished C. viridocoeruleus var. pervelatus. The morphological separation of C. viridocoeruleus s.l. from C. mahiquesii is based on the following characters: i) epigeous growth; ii) different habitat (nemoral or sclerophyllous broad-leaved or coniferous woodlands); iii) a variable, not farinaceous smell; and iv) smaller spores (i.e. 9.2–10.4 × 5.4–6 μm, Consiglio et al. 2007a). Fig. 1 shows the clear differentiation of C. mahiquesii from C. anserinus and C. viridocoeruleus s.l. from a molecular point of view, since the mean genetic distances between these species are 5.6 % and 5.4 %, respectively.

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