Two new species of *Cortinarius* collected under *Quercus rotundifolia* in the Mediterranean area of southern Spain

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Two new species of the genus *Cortinarius* (subgenus *Phlegmacium*) are proposed: *C. inusitatus* (sect. *Claricolores*; clade *Phlegmacium*, subclade *Glaucopodes*) and *C. viscidoamarus* (sect. *Phlegmacium*; clade *Percomes*). These species were collected in the holm oak forests of southern Spain. Morphological, macrochemical and molecular (internal transcribed spacer sequences) data of these species are given. Scanning (FESEM) micrographs of the spores and color pictures of the basidiomes *in situ* are provided.

Key words: *C. inusitatus*, *C. viscidoamarus*, DNA sequences, Granada, holm oak, macrofungi, morphology, *Phlegmacium*, taxonomy, systematics

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Introduction

Several studies have examined the genus *Cortinarius* from the Mediterranean area of Europe (*e.g.* Malençon and Bertault, 1970; Chevassut and Henry, 1975, 1982; Gutiérrez and Vila, 2001, 2002; Vila and Llimona, 2002, 2006; Frøslev *et al.*, 2006; Ballarà *et al.*, 2007; Ortega *et al.*, 2007, 2008). They mainly focused on species collected from thermophilous habitats (*e.g.* holm oak woodlands and *Cistus* shrub communities). Several new taxa have been described, including many *Phlegmacium* species that occur in southern European broadleaf forests in basic and acid soils. These Mediterranean forests are representative of the Iberian Peninsula and are rich in *Cortinarius* species (Mahiques, 1999, 2001, 2002, 2004, 2006). We present two new species collected from *Quercus rotundifolia* woodland (= *Q. ilex* subsp. *ballota*) in Granada province (Andalusia, southern Spain): *Cortinarius inusitatus* and *C. viscidoamarus*. The study area was a 3,000 m^2 site in the Sierra de Huétor Natural Park (NW of Granada), at 1,200-1,300 m.a.m.s.l. The soil there is basic, the thermotype is meso-supramediterranean, and the ombrotype is dry-subhumid (*Berberidio hispanicae-Querceto rotundifoliae S*) (Valle, 2003). In some places, decarbonated outcrops are colonized by communities of *Cistus laurifolius*. In addition, several *Pinus sylvestris*, *P. nigra* subsp. *salzmannii* and *P. halepensis* trees had grown as a result of past reforestation schemes. However, these pines were recently uprooted in an attempt to promote the regeneration of native vegetation.

In *Quercus rotundifolia* forests, the macrofungal fruiting period is from the middle of October to the middle of January. Ortega and Navarro (2006) and Ortega and Lorite (2007) reviewed the mycobiota of Andalusian holm oak forests. Some interesting species of *Phlegmacium* collected from the same locality include: *Cortinarius alcalinophilus* Rob. Henry, *C. caesiocortinatus* Jul. Schäff., *C. caligatus* Malençon, *C. calochrous* (Pers.) Gray, *C. cedretorum* Maire, *C. haasii* var. *quercus-ilicicola* A. Ortega, Suár.-Sant. & J.D. Reyes, *C. nanceiensis* var. *bulbopodius* Chevassut &

Rob. Henry, *C. parasuaveolens* (Bon & Trescol) Bidaud, Moënne-Locc. & Reumaux, *C. platypus* (M.M. Moser) M.M. Moser, *C. rickenianus* Maire, *C. selandicus* Frøslev & T.S. Jeppesen, *C. splendidior* Bidaud, *C. subgracilis* Moënne-Locc. and *C. variiformis* Malençon.

Cortinarius viscidoamarus and *C. inusitatus* are common in the study area and are characterized by the following. *Cortinarius inusitatus* has abundant universal veil remnants on the cap surface and a bright color (violet, pinkish and yellowish olive hues). *Cortinarius viscidoamarus* has a pallid cap, and is easily recognized by the glutinous, bitter tasting, yellowish universal veil on the cap and stipe surface.

The almost exclusive use of morphological and ecological data sets for species delimitation in *Cortinarius* has created confusion over taxonomy and nomenclature. The incorporation of molecular data into the systematics of the genus, in combination with other data sets, has enabled the taxonomic delimitation of numerous species and has led to an assessment of the morphological characteristics used in the taxonomy of this genus. Thus, the usefulness of sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA (nrDNA) has been demonstrated in many studies (Liu *et al*., 1995, 1997; Seidl, 2000; Peintner *et al*., 2003; Garnica *et al*., 2003, 2005; Kytövuori *et al*., 2005; Frøslev *et al*., 2006, 2007). Furthermore, the use of ITS as species-identifier sequences (barcoding) in *Cortinarius* has been proposed (Frøslev *et al*., 2007; Ortega *et al*., 2008). In this paper, we describe two new species of *Cortinarius* subg. *Phlegmacium*. The description of these species involved macromorphological and micromorphological analyses, as well as a phylogenetic analysis of ITS sequences.

Materials and methods

The classification of *Cortinarius* follows Brandrud *et al*. (1994), Frøslev *et al*. (2005) and Garnica *et al*. (2005). All the material was deposited in the herbarium GDA (University of Granada, Spain). The macromorphological analyses were done on both young and mature fresh basidiomes. We observed the pileus, stipe and gill color *in situ* (Kornerup and Wanscher, 1973) and recorded the smell and taste. The KOH (25-40%) reaction was performed on the pileus, stipe, bulbipellis and context. The habit variability was illustrated in color photographs (Figs 3 a-a', 3 b-b').

The micromorphological analyses were carried out on free-hand radial sections of pileus and longitudinal sections of the gills mounted in KOH (2-3%) and Congo red (in 2% NH_4OH). Twenty spore measurements of several basidiomes were made for each population. With these data the minimum, mean (underlined in the text) 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) in the "Servicio Técnico de Apoyo a la Investigación" of the University of Granada. FESEM spore observation was carried out according to the method of Ojeda (1997) as follows. (i) Spore hydratation: the sample was placed in ammonia water [five drops of ammonia (32%) per 1.5 ml of water], for four or five days at room temperature. (ii) Sample fixation: the sample was placed in 1:1 glutaraldehyde (2.5%), osmium tetroxide (1%) and cacodylate buffer 0.1 M, pH 7.4, at 4ºC for four hours. (iii) Sample washing: the sample was first washed with cacodylate buffer (five ten-minute washes), then with distillated water (three five-minute washes) at room temperature. (iv) The sample was kept in ethanol (50, 70, 90 and 3 × 100 %) for fifteen minutes at room temperature. (v) At the critical point, carbon dioxide was added according to Anderson (1951), in a Polaron CPD 7501 dryer. (vi) The sample was metalized in gold according to the ion sputtering method, using a Polaron Unit SEM Coating E5000 metallizer.

For the molecular analysis, the total genomic DNA was extracted using the CTAB method (Doyle and 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 and Zimmer, 1996). The PCR reactions were performed in a volume of 50 µl under standard conditions (Innis *et al*., 1990). Automated sequencing of the purified PCR products was performed in both directions using the ITS-5 and C26A primers on a 3100-Avant Genetic Analyzer. Nucleotide sequences were edited with the SEQMAN II v. 3.61 program from the DNASTAR software package (LASERGEN). The ITS region was amplified for all of the collections in the study (three for *C. inusitatus* and eight for *C. viscidoamarus*). BLAST searches were carried out with the ITS sequences of both species, to search for highly identical sequences. The most similar sequences were aligned with the sequences of each one of the new species. In both cases, a neighbor-joining tree was constructed (using the program MEGA 3.1; Kumar *et al*., 2004; data not shown) to detect the sequences that were most closely related to the ITS of the new species. Only these sequences were used in the final alignment, which is available from TreeBASE (study accession number S2309). The genetic distances (*p*-distance) between the sequences were calculated using MEGA 3.1. The unrooted neighbor-joining tree derived from MEGA 3.1 (gaps were treated as missing data), showing the relationships between *C. inusitatus* and *C. viscidoamarus* and their most closely related species, is included in Fig. 1. EMBL accession numbers for the sequences of the two new species are shown in the Taxonomy section, and the GenBank accession numbers for the remaining species are shown in Fig. 1.

Taxonomy

Cortinarius inusitatus A. Ortega, Bidaud, Suár.-Sant. & Vila, **sp**. **nov**.

(Figs 2a, 3a-a')

MycoBank: MB 512101.

Etymology. Latin: *inusitatus*, "unaccustomed"; with unusual characteristics.

Pileus 40-120 mm latus, (hemi-)sphaericus vel applanatus, interdum centro leviter depresso; glutinosus; violaceo-rubeolus vel griseo-violaceus, ad marginem olivaceo-luteus, in vetustis basidiomatibus roseo-griseolus vel cremeo-griseolus. Velum universale copiosum, album, caeruleum vel griseolum, lutescente in vetustis speciminibis, fractum in plurimas cremeo-roseas vel cremeo-luteolas squamas, membranaceas, in pileo et in bulbi exteriore parte. *Lamellae* albidae vel cremeo-roseae. *Stipes* 30-100 × 20-30 mm, bulbo manifesto (26-40 mm), albidus, roseo colore leviter suffusus. Cortina cremeo-rosea. *Caro* albida, in vulneribus et vetustis speciminibus lutescente. Odor atque sapor inconspicui. *Sporae* 8.8-10.8 × 4.5-5.6 µm, ellipsoideae, subamigdaliformes vel amigdaliformes, mediocriter verrucosae. ***Holotypus***, in Hispania, Granada, prope Huétor, Santillán (Natural Park of Sierra de Huétor), 26.11.2006, sub Cisto laurifolio, A. Ortega lectus, GDA 53699.

Section *Claricolores* Kühner & Romagn. ex Moënne-Locc. & Reumaux (Brandrud *et al*., 1994).

Clade *Phlegmacium*, subclade *Glaucopodes* (Frøslev *et al*., 2005).

Pileus 40-120 mm, (hemi) spherical, convex, plano-convex, plane, sometimes slightly depressed in the centre with age; margin entire, involute then straight; reddish violet (14C3, 10C4, 11C4) or grayish violet (18C2), with yellowish olive (3C3) hues towards the margin, the older basidiomes progressively turn grayish pink (7B2), grayish cream (5B4) or grayish orange (6C2), the dried material uniformly and intensely yellows (4A6). *Universal veil* abundant, white, with bluish (23A2) and/or grayish orange (6C2) hues. The veil yellows (2B2) in older specimens, breaking into numerous cream pink (6A2) or cream yellow (5A2-3) membranous patches or scales on the pileus and the bulb surface. *Lamellae* crowded, adnate, sinuate or emarginate, whitish with cream pink (6A2) and pale grayish pink (7B2) hues, edges entire or serrulate concolor or slightly paler. *Stipe* 30-100 × 20-30 mm, as short or as long as the pileus diameter with an evident but scarcely marginate bulb (26-40 mm), whitish with a slight pinkish shade (7B2). *Cortina* (*partial veil*) a cream pink shade (6A2) abundant on bulb, for this reason, the bulb surface sometimes take on a cream pink color. *Context* whitish, but yellow in older and damaged specimens. *Smell and taste* not distinctive.

Chemical reactions KOH (30%) brick red or reddish brown (7D7, E7, 8D7) on the cap and the stipe surface, yellowish orange (5B3) in the context.

Spores 8.8-$\underline{9.2}$-10.8 × 4.5-$\underline{5.1}$-5.6 µm, ellipsoid, subamigdaliform or amigdaliform (Q: L/w = 1.6-$\underline{1.8}$-1.96), with moderate ornamentation and ± anastomosing warts. *Basidia* tetrasporic (25-40 × 8-10 µm). *Lamellar edges* with rare, cylindric-claviform, basidioloid, hyaline, 7-10 µm wide marginal cells. *Hyphae of universal veil* hyaline or slightly yellowish (in older basidiomes), 2.5-5 µm wide. *Pileipellis* duplex. *Epicutis* thick, the upper layer gelatinous, hyphae erect or ± sinuous,

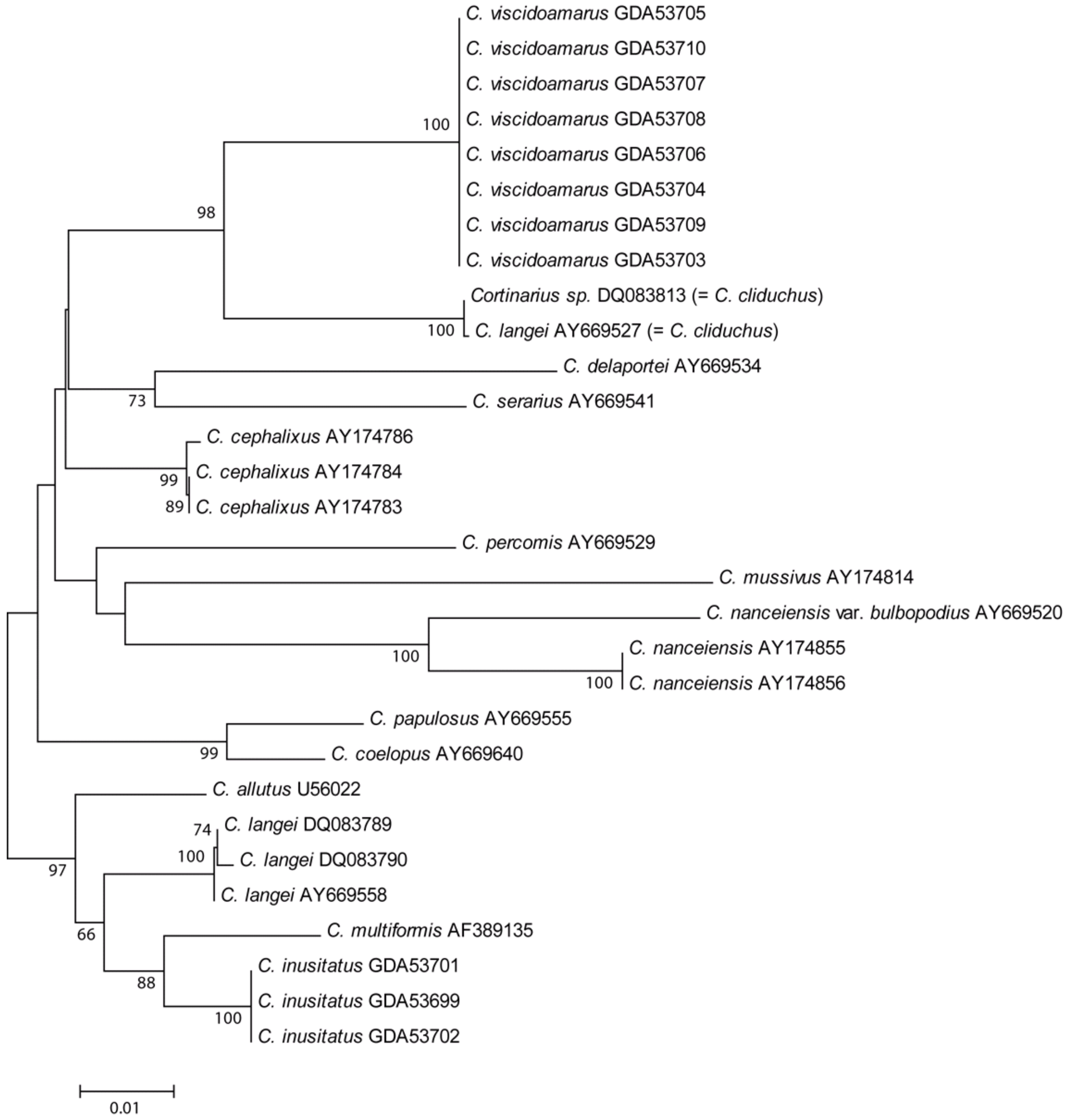

Fig. 1. Unrooted neighbor-joining tree showing the relationships between *Cortinarius inusitatus* and *C. viscidoamarus* and their most closely related species. Bootstrap values ≥ 50% are shown above the branches. The accession number (for the sequences taken from GenBank) or herbarium references (for the sequences generated in this study) are shown after each taxon name.

cylindrical, 2-4(-5) µm wide, colorless or with a slight to moderate amount (rarely high in older basidiomes) of yellow vacuolar and epiparietal-encrusted pigment. *Hypocutis* a dense interwoven repent layer, hyphae 8-22 µm wide, with abundant transverse septa delimiting short or moderate length cellular elements (± subcellular structure), forming a compact lower layer with yellowish vacuolar and parietal pigment. *Clamp connections* present in all tissues.

Habitat: in sclerophyllous Mediterranean holm oak forest and *Cistus* shrubs, under *Q. rotundifolia* and *C. laurifolius*.

Known distribution: the Iberian Peninsula (type locality).

Material examined: Spain, Granada, Huétor Santillán, the Sierra de Huétor Natural Park, forest track of the Pajareras, 0.5 Km, 1,250 m, under *Quercus rotundifolia*, 26 November 2006, A. Ortega (GDA 53699; **HOLOTYPE**), EMBL accession number: FM202128; *idem*, under *Cistus laurifolius*, 2 December 2006, A. Ortega (GDA 53701), EMBL accession number:

FM202129; *idem*, 9 December 2006, A. Ortega (GDA 53702), EMBL accession number: FM202130.

Notes: *Cortinarius inusitatus* is distinguished by: (i) the bright, reddish violet color of the pileus, which turns progressively grayish pink, cream or orange and yellowish or yellowish olive towards the margin; (ii) the presence of numerous white scales or patches on the bulb surface and especially on the cap surface of younger specimens, which is why it resembles several *Amanita* species (e.g. *A. junquillea*); (iii) the slight marginate bulb; (iv) the gills and stipe with no violet or bluish hues; (v) the context that becomes yellow in older basidiomes and turns yellowish orange with KOH; (vi) the pileus surface that is reddish brown or brick red with KOH.

The molecular analysis showed *C. multiformis* Fr. *sensu* Moser *et al*. (2002) and Peitner *et al*. (2004), *Cortinarius langei* Rob. Henry *sensu* Garnica *et al*. (2005) *pro parte* (coll. TUB 011861) (= *C. xantho-ochraceus* P.D. Orton; see Münzmay and Saar, 2005), and *C. allutus* Fr. *sensu* Liu *et al*. (1997) as the species most closely related to *C. inusitatus* (Fig. 1). However, the high genetic distances between the ITS of *C. inusitatus* and the sequences of the other two species (p = 0.025 with *C. multiformis*, and p = 0.028 with *C. langei* sequences), together with the homogeneity of the intraspecific ITS of *C. inusitatus* (100% similarity), support the specific identity of *C. inusitatus*. Various studies have shown the effectiveness of ITS sequences in identifying *Cortinarius* species (Frøslev *et al*., 2007; Ortega *et al*., 2008), where genetic distances of approximately 2% indicate a specific difference. This molecular analysis result is consistent with the morphology, as both *C. multiformis* and *C. langei* are very different, morphologically, to *C. inusitatus*.

According to Frøslev and Jeppesen (The *Phlegmacium* website) *Cortinarius langei* (voucher TF1999-084) is a rare species; “it has a pale yellowish brown cap (often with patches of universal veil), pale gills and a broadly marginate bulb. The spores have the net-like ornamentation characteristics of section *Calochroi*, and no hypoderm is present”. It seems clear that *C. inusitatus* does not corresponds to *C. langei sensu* Frøslev and Jeppesen (voucher TF1999-084, JV-01-642), since our material has (i) evident reddish violet hues on the cap, (ii) no broadly marginate bulb, (iii) smaller spore ornamentation and (iv) a defined hypoderm. The morphological relationships of *C. inusitatus* with *C. allutus* are limited, since *C. allutus* has a different habit, with no marginate bulb, and a lack of reddish-violet hues in the cap and scales pileus (present in *C. inusitatus*). Moreover, *C. allutus* is found under *Picea* in north Europe, and therefore has a different habitat to *C. inusitatus*, Finally, the differentiation between *C. inusitatus* and *C. multiformis* (coll. IB 198000618) is clear. In spite of sharing a similar habitat, they are very different morphologically (as can be seen in the color photograph published by Moser *et al*., 2002: *Cortinarius* 125). Thus, *C. multiformis* has a yellow or yellowish orange pileus (reddish violet or grayish violet in *C. inusitatus*), with no reddish violet hues (present in *C. inusitatus*) and very poor universal veil development (abundant in *C. inusitatus*). In addition, *C. multiformis* has smaller spores (7.5-$\underline{8.6}$-9.5 × 4.2-$\underline{4.6}$-5.4 µm) than *C. inusitatus* (8.8-$\underline{9.2}$-10.8 × 4.5-$\underline{5.1}$-5.6 µm).

With regards to the habit of *Cortinarius inusitatus*, there are references in the literature to two morphologically close Mediterranean species: *C. caeruleo-ochrascens* Chevassut & Rob. Henry (not *sensu* Moser, 1997) and *C. rioussetorum* Bidaud, Moënne-Locc. & Reumaux. According to Chevassut and Henry (1975), the first species has: (i) a similar morphology and a thick but scarcely marginate bulb; (ii) grayish blue or violet lilac hues in the cap, which soon turn yellowish ochre; (iii) abundant cortina on the bulb surface; and (iv) a whitish context, which turns slightly brown when cut. However, our species has important differences. With regard to *C. caeruleo-ochrascens*: (i) in *C. inusitatus* the cap and bulb scales are more evident and have a membranous-granulate consistency, while the cap scales are fibrillose in *C. caeruleo-ochrascens*, (ii) the yellowing in older caps is different (yellow, yellowish olive in *C. inusitatus vs*. ochre or ochre brown in *C. caeruleo-ochrascens*), (iii) the spore shape is similar (Moser, 1997), but the spore size

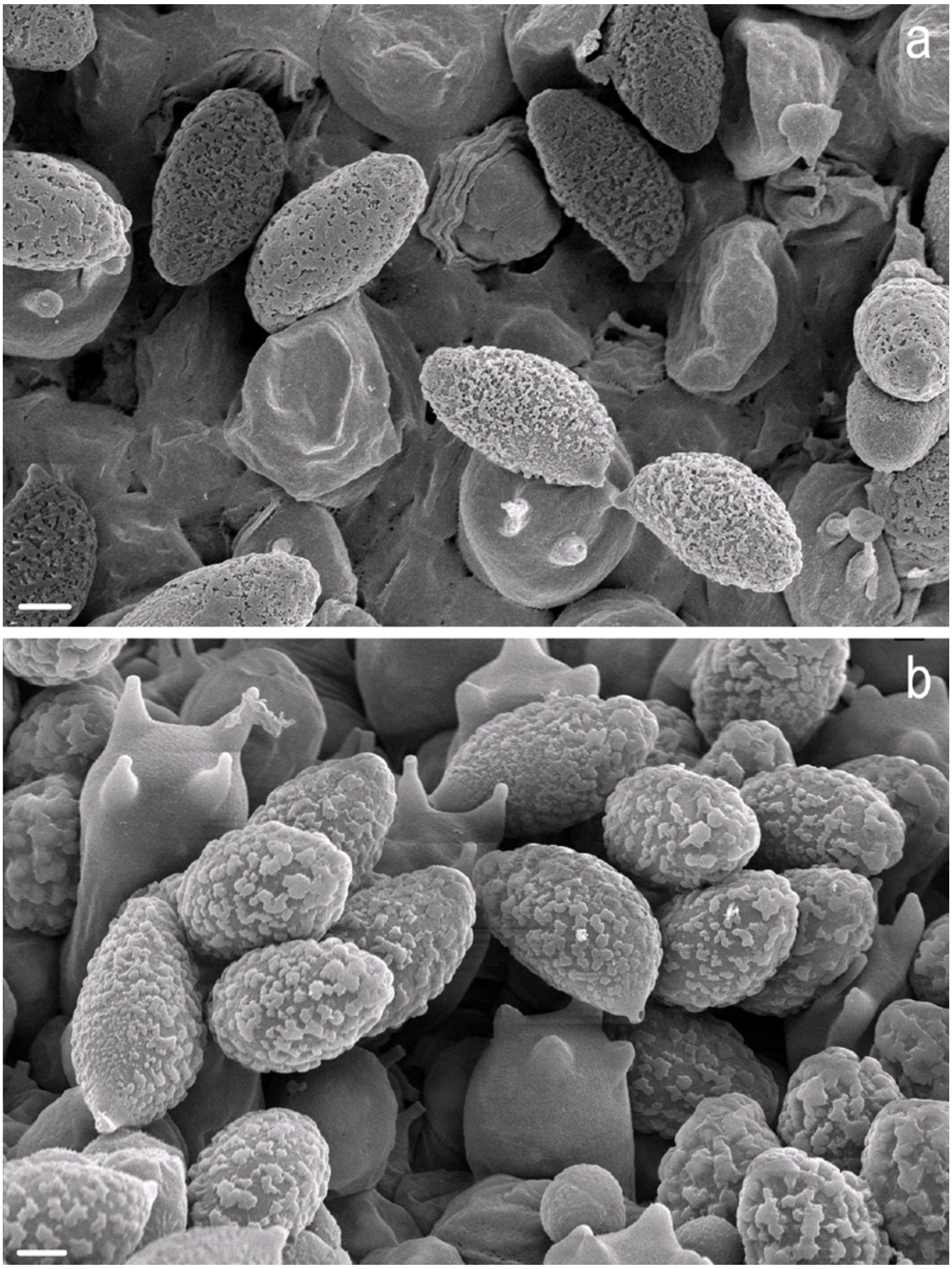

Fig. 2. Scanning (FESEM) micrographs of the spores. **a)** *Cortinarius inusitatus* (holotype, GDA 53699). **b)** *C. viscidoamarus* (holotype, GDA 53709). Bars = 2.2 µm.

[8.8-9.2-10.8 × 4.5-5.1-5.6 µm *vs*. 10.8-14.5 × 7.2-8.3 µm (Chevassut & Henry, *loc. cit.*) or 9.4-10.2-11.2 × 5.3-5.8-6.2 µm (Moser, *loc. cit.*)] and spore ornamentation (moderate, *vs*. coarsely warted) are different, (iv) the KOH reaction in the cap is different [brick red or reddish brown for *C. inusitatus vs*. negative (ochre brown) for *C. caeruleo-ochrascens*].

Cortinarius rioussetorum, according to Bidaud *et al*. (2000), has (i) a violet lilac pileus that soon discolors, becoming yellow or yellowish ochre, (ii) abundant whitish granulate scales formed by universal veil remnants and (iii) amigdaliform to citriform spores. Our species is clearly different, since *C. rioussetorum* has violet gills and stipe, an abruptly marginate bulb and wider [(8)9-11.5(12) × 5.5-6(6.5) µm *vs*. 8.8-11.2 × 4.5-5.6 µm] and strongly warted spores. According to Frøslev *et al*. (2008, in preparation), *C. rioussetorum* belongs to section *Calochroi* and contains anthraquinonoid pigments in low quantities.

Fig. 3. Basidiomes in their own habitat. **a-a')** *Cortinarius inusitatus*. **b-b')** *C. viscidoamarus*.

Cortinarius viscidoamarus A. Ortega & Suár.-Sant., **sp**. **nov**.

(Figs 2b, 3b-b')

MycoBank: MB 512102.

Etymology: Latin: *viscidus*, "glutinous", *amarus*: "bitter"; for the bitter viscosity of the pileus surface.

Pileus 50-80 mm latus, (hemi)sphaericus vel applanatus, centro leviter depresso, margine involuto dein recto; glutinosus, mox paene siccus; primo marginem versus albidus, in reliqua parte pallide luteus, cremeo-ochraceus, dein omnino luteus vel cremeo-ochraceus; pileocutis separabilis. Velum universale glutinosum, pallide luteum. *Lamellae* albidae deinde cremeo-luteae vel cremeo-ochraceae, acies integra vel renulata. *Stipes* 50-100 × 10-18 mm, crassus, cylindraceus vel ventricoso-inflatus, rigidus, bulbo clavato vel submarginato (× 15-28 mm), albidus, luteis universalis veli reliquis obtectus. *Caro* albida, leviter brunnescens in vetustis basidiomatibus; odor atque sapor inconspicui, pileocutis lentitia subamara. *Sporae* 11.2-13.8 × 6.2-7.2 µm, ellipsoideae, subamigdaliformes vel amigdaliformes, mediocriter verrucosae. ***Holotypus***, in Hispania, Granada, prope Huétor, Santillán (Natural Park of Sierra de Huétor), 5.1.2007, sub *Quercus rotundifolia*, A. Ortega lectus, GDA 53709.

Section *Phlegmacium* (Fr.) Gillot & Lucand (Brandrud *et al*., 2004).

Clade *Percomes* (Frøslev *et al*., 2005; Garnica *et al*., 2005).

Pileus 30-60(-70) mm, (hemi)spherical, convex, plano-convex, plane or slightly depressed in the centre with age; margin entire, involute then straight; in the younger basidiomes, normally, whitish towards the margin, pale yellow (3A3), light yellow (4A4), pastel yellow (4A5), cream ochre (5B4), light orange (5A5) or brownish orange (6C8) in the disc, uniformly yellowish, cream ochre or orange in older specimens; cuticle easily peeled, glossy and distinctly viscid, soon almost dried. Pale yellow (4A3) or light yellow (3-4A4) glutinous universal veil remnants present, but not abundant. *Lamellae* moderately crowded, adnate or emarginate with a decurrent tooth, whitish, sometimes with very faint pinkish lilac (7-8B2) hues, then yellowish cream (5A3-4), yellowish (4A4-5) or cream ochre (5B4-5) with or without clay pinkish (6B3) hues, edges entire or crenulated, concolors or slightly paler. *Stipe* variable, 75-85 × 10-12 mm, 40-60 × 9-20 mm, or 30-50 × 30-40 mm, as long as, shorter, or longer than the cap diameter, cylindric, clavate to robust-swollen, rigid, bulb 12-28 (-45) mm in diam., ± evident, clavate or more frequently submarginate, and ± atenuate at base, whitish with yellowish (4A4-5) universal veil remnants present, but not forming distinct girdles or scales. *Context* whitish, slightly browning (5B4-5), especially towards the base. *Smell and taste* not distinctive except pileus glutin, which is bitterish.

Chemical reactions KOH (30%) cream ochre (5B4-5) on cap and stipe; negative on context.

Spores 11.2-<u>11.9-12.4</u>-13.8 × 6.2-<u>6.6-6.8</u>-7.2 µm, ellipsoid, subcylindrical, ovoid-amigdaliform, subamigdaliform or amigdaliform (Q: L/w = 1.6-<u>1.8-1.84</u>-2.1), with moderate ornamentation formed by ± anastomosing warts. *Basidia* tetrasporic, cylindrical-claviform (25-38 × 8-10 µm). *Lamellar edges* with rare, basidioloids, cylindrical or claviform, hyalines, 7-9.5 µm wide marginall cells. *Universal veil* remnants of ± thick-walled hyphae, 2.5-5 µm wide, with an intense yellowish vacuolar, sometimes epiparietal-encrusted pigment. *Pileipellis* simplex. Epicutis thick, the upper layer gelatinous, hyphae 2.4-7.5 µm wide, erect, ± sinuous and some winding, with cylindrical or slightly acute to clavate terminal cells, colorless or pigmented with a slight, moderate or strong (depending on the intensity of the cap surface color) yellowish vacuolar and/or epiparietal-encrusted pigment. Lower layer formed by irregularly interwoven repent, colorless or pigmented, slightly thick-walled, hyphae 5-12 µm wide, forming a compact (sub)-cellular layer, with yellowish vacuolar and parietal pigment. Clamp connections present in all tissues.

Habitat: in sclerophyllous Mediterranean holm oak forest, under *Quercus rotundifolia* on basic soil.

Known distribution: Iberian Peninsula (type locality).

Material examined: SPAIN, Granada, Huétor Santillán, Sierra de Huétor Natural Park, road track of the Pajareras, Km 0.5, 1,250 m, under *Quercus rotundifolia*, on basic soil, 17 December 2005, A. Ortega (GDA 53703), EMBL accession number: FM202131; *idem*, 26 November 2006, A. Ortega (GDA 53704), EMBL accession number: FM202132; *idem*, 2 December 2006, A. Ortega (GDA 53705), EMBL accession number: FM202133; *idem*, 9 December 2006, A. Ortega (GDA 53706), EMBL accession number: FM202134; *idem*, 22 December 2006, A. Ortega (GDA 53707), EMBL accession number: FM202135; *idem*, 29 December 2006, A. Ortega (GDA 53708), EMBL accession number: FM202136; *idem*, 5 January 2007, A. Ortega (GDA 53709; **HOLOTYPE**, isotype in GARN-9377), EMBL accession number: FM202137; *idem*, 11 November 2007, A. Ortega (GDA 53710), EMBL accession number: FM202138.

Notes: *Cortinarius viscidoamarus* is distinguished by: (i) the pileus color, withish, yellowish, yellowish cream or orange towards the disc, uniformly yellowish, cream yellow, cream ochre or orange in mature basidiomes; (ii) the bitterish cap surface, with distinctly viscid fresh, becoming dry; (iii) the distinct and submarginate bulb; (iv) sometimes, pinkish lilac hues present on gills, absent on stipe and context; (v) yellowish universal veil remnants present on cap and stipe; (vi) large spores: 11-13.8 × 6.2-7.2 µm; (vii) fruiting in Mediterranean subhumid sclerophyllous *Quercus rotundifolia* woodlands.

While the aforementioned features characterize *C. viscidoamarus*, some morphological variation has been observed among the *C. viscidoamarus* collections. Specimens of some collections have a cylindrical slender stipe, while specimens of others show a clavate or robust stocky stipe. Moreover, the gill color differs between specimens of the same collection, as does the pileus color, which varies from pallid yellow to brownish orange. However, this morphological variation between and within *C. viscidoamarus* collections is not correlated with the molecular data; as the ITS sequences of the eight collections are identical (*p* = 0.000). Based on this result, we consider that these morphological characteristics are not taxonomically significant, although they are not always consistent with the literature (*e.g.* Bidaud *et al.*, 1999; Consiglio *et al.*, 2007), in which, in some instances, the presence of pinkish hues on the gills is used to separate taxa.

Cortinarius viscidoamarus is morphologically similar to *C. cliduchus* Fr. (= *C. vitellinopes* Secr. ex Gillet), which grows in frond (broadleaf) and coniferous forests in nemoral zones (Münzmay and Saar, 2005). According to Bidaud *et al.* (1999) and Consiglio *et al.* (2005), *C. vitellinopes* has smaller spores (8-11 × 5.5-6.5 µm *vs.* 11.2-13.8 × 6.2-7.2 µm). However, Chevassut and Henry (1982) suggest a variable spore size for this species (*i.e.* 9.4 × 4.7 µm and 13.7 × 6.5 µm). An important study of *C. cliduchus* was carried out by Münzmay and Saar (*loc. cit.*), in which they described and discussed the taxonomic position of this rare species. Our material is certainly closely related to *C. cliduchus*. However, it can be easily distinguished by the following: (i) a different pileus color, which is yellowish orange *vs.* yellow ochre or yellowish; (ii) bitterish pileus glutin, which is not found in *C. cliduchus*; (iii) larger spores: 11.9-12.4 × 6.6-6.8 µm *vs.* 9.8-10.9 × 6.1-6.3 µm (mean values); (iv) smaller spore ornamentation; and (v) a different habitat: sclerophyllous *Quercus* forests *vs.* nemoral forests (*C. cliduchus*).

Cortinarius cephalixus Secr. ex Fr. *sensu* Bidaud and *C. cephalixus* var. *subopimatus* Bidaud, Moënne-Locc. & Reumaux are two variants that differ from each other in their stipe morphology and spore size (Bidaud *et al.*, 1999; Consiglio *et al.*, 2005, 2007). Despite both taxa being morphologically similar to *C. viscidoamarus*, they are clearly different. Our species has (i) a different cap and lamellae color (greenish yellow hues in *C. cephalixus*); (ii) bitterish viscosity of the pileus surface; (iii) a universal yellow veil with no olive hues; (iv) scarcer and less evident universal veil remnants on the stipe, which do not form girdles or scales (present in *C. cephalixus*); and (v) frequently has submarginate bulbs.

From a molecular point of view, *Cortinarius viscidoamarus* is clearly differenttiated from the most closely related species, according to the nucleotide databases: *C. cliduchus* [= *Cortinarius sp.* (coll. JV 01-574, sequence DQ083813) (Frøslev *et al.*, 2008, in preparation); = *C. langei* Rob. Henry *sensu* Garnica *et al.* (2005; coll. TUB 0011860, sequence AY669527)] (*p* = 0.049, 29 nucleotide changes and six gaps from one to three base pairs). The morphological relationship between *C. viscidoamarus* and *C. cephalixus* is clearly not supported by the molecular data, since the mean genetic distance between both species is 5.26%.

Cortinarius decurtatus Rob. Henry and *C. aurantiopallidus* Bidaud are two taxa which are morphologically similar to *C. viscidoamarus* and grow in the same habitat. However, our species is easily differentiated from them, since the habit of both *C. decurtatus* and *C. aurantiopallidus* differs from that of *C. viscidoamarus*. According to Bidaud *et al.* (1999), these species have a massive pileus (up to 100 mm wide *vs.* 50-80 mm) and stocky basidiomes, and the yellowish, glutinous universal veil remnants on the cap and stipe surface are absent. *Cortinarius decurtatus* has shorter (i.e. 8-11.5 × 6-7 µm *vs.* 11.2-13.8 × 6.2-7.2 µm) spores than *C. viscidoamarus*, with a different shape (Q = 1.5 *vs.* 1.8-1.84) (see Bidaud *et al.*, *loc. cit.*; Henry, 1989). *Cortinarius aurantiopallidus* has some tiny flat scales towards the centre of the pileus that are not seen in *C. viscidoamarus*.

Recently, Ortega and Reyes (2005) studied material identified as *Cortinarius caesiostramineus* var. *cadinanos-aguirrei* Moënne-Locc. & A. Ortega from the Jaén province in Spain. This material has several morphological characteristics that are relatively similar to *C. viscidoamarus*.

However, the spores are smaller: 8.5-9.5-11 × 5.2-5.6-6 µm *vs.* 11.2-11.9-12.4-13.8 × 6.2-6.6-6.8-7.2 µm. A molecular analysis of the Jaén material of *C. caesiostramineus* var. *cadinanos-aguirrei* revealed that it corresponds to a whitish cream gill form, similar to *C. variiformis* Malençon (Garnica, pers. comm.).

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