Morphological and ITS identification of *Cortinarius* species (section *Calochroi*) collected in Mediterranean *Quercus* woodlands

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Species of *Cortinarius* sect. *Calochroi* show a high grade of morphological variability in the Mediterranean region with regard to vicariant populations in the Eurosiberian region. This has contributed to confusion on taxonomy and nomenclature, especially when the variability affects the characters traditionally used for taxonomic delimitation. In this paper, we have attempted to clarify the taxonomy and nomenclature of several *Calochroi* taxa in the Mediterranean area by using macromorphological and spore analyses, as well as phylogenetic analysis of ITS sequences. We have demonstrated the usefulness of ITS sequences for species rank identification in *Cortinarius* (barcoding). We have been able to assign the studied material to eight species using ITS sequences (*C. haasii*, *C. parasuaveolens*, *C. platypus*, *C. rickenianus*, *C. sancti-felicis*, *C. selandicus*, *C. splendidior* and *C. subgracilis*), and to detect misidentified collections that were used by their authors to describe a new species (*C. violaceipes*). Moreover, molecular data have been useful to test the taxonomic value of morphological and spore characters. The morphological analysis has enabled us to detect morphological variability previously unreported in several taxa. A new variety, *C. haasii* var. *quercus-ilicicola*, is proposed on the basis of morphological and molecular data. Also, the taxonomic status of *C. rickenianus* is discussed. Finally, our results increase both the distribution area as well as the ecological preferences of five studied species, and several important biogeographical disjunctions are reported (*C. selandicus* and *C. subgracilis*).

Key words: *Cortinarius*, FESEM, ITS sequences, morphological and spore diversity, sclerophyllous forests

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Introduction

The Mediterranean basin is the largest of the world's five Mediterranean-climate regions. It surrounds the Mediterranean sea, stretching west to east from Portugal to Jordan (Lebanon, Israel) and north to south from northern Italy and Slovenia to Morocco (Médail and Quézel, 1999). The Mediterranean region harbours a high degree of biological diversity, being considered one of the 25 biodiversity hotspots of the world (Myers *et al.*, 2000). This high biodiversity level is closely associated with the great ecological heterogeneity of the Mediterranean region and its complex historical changes.

Fungal diversity in the Mediterranean basin is high, and the presence in this area of populations of taxa differing morphologically with respect to their northern vicariants is frequent. Consequently, many taxonomic proposals have been made in order to explain Mediterranean fungal variability. This situation is frequent in the case of the genus *Cortinarius*, and specifically in the taxa of section *Calochroi* M.M. Moser & E. Horak (Frøslev *et al.*, 2006, 2007).

Calochroi species show high variability in Mediterranean *Quercus* woodlands (*Q. ilex* subsp. *ballota, Q. ilex* subsp. *ilex*, *Q. coccifera*, etc.) with regard to vicariant populations in the Eurosiberian region growing under broadleaf trees and/or conifers. Different interpretations that mycologists offer for this morphological variability between the Mediterranean *vs.* central-European populations have resulted in confusion concerning taxonomy and nomenclature, especially when the variability affects the characters traditionally used for taxonomic delimitation (*e.g.* spore size; Clemençon, 1979). The incorporation of molecular data to the systematics of the genus *Cortinarius* has enabled not only clarification of interspecific phylogenetic relationships, but also the taxonomic delimitation of numerous species, and has made it possible to evaluate the taxonomic value of morphological characters used in the taxonomy of this genus. In this sense, the usefulness of the sequences of the internal transcribed spacers (ITS) of the nuclear ribosomal DNA (nrDNA) have been shown in many studies (Liu *et al*., 1995, 1997; Seidl, 2000; Peintner *et al*., 2003, Kytövuori *et al*., 2005; Frøslev *et al*., 2006, 2007), and they have been proposed as species-identifier sequences (barcoding) in *Cortinarius* (Frøslev *et al*., 2007). In this paper, we have attempted to clarify the taxonomy and nomenclature of several taxa of *Cortinarius* sect. *Calochroi* in the Mediterranean area. To this end we performed macro- and micromorphological analyses, and phylogenetic analysis of ITS sequences.

Materials and methods

The studied material has been collected from localities of Granada and Jaén provinces (Andalusia, southern Spain). Vouchers are deposited in the University of Granada herbarium (GDA-GDAC). We have also studied material for comparison from the herbaria of CUSSTA, Córdoba (JA-Cussta), herbarium of Institute of Microbiology of Innsbruck, Austria (IB), and personal herbaria of A. Bidaud (AB), J.A. Cadiñanos Aguirre (CAD), Dr. Garcia Bona (MN) and R. Mahiques (MES). For colour definition, we relied on Kornerup and Wanscher (1973). KOH (25-40%) macrochemical reactions were tested on dried material. Microscopic examinations were made with a Zeiss optical microscope (× 2000). Where possible, for each population, we measured 20 spores × 3 basidiomata and calculated the range of values, including the mean, as well as the length:width ratio (Q: L/W). The spores were morphologically studied using a Leo (Zeiss), model 1539 Gemini, Field Emission Scanning Electron Microscope (FESEM) in the Servicio Técnico de Apoyo a la Investigación of the University of Granada. We also analysed a character that could be used to differentiate *C. molochinus* Bidaud & Ramm from *C. nymphicolor* Reumaux, based on our own material together with data from the literature (Moser and Jülich, 1986; Bidaud *et al*., 1993, 2001; Cadiñanos-Aguirre, 1995; Soop, 2005). This character is the degree of slenderness, defined by three parameters: (i) Ratio between pileus diameter (P.D.) and stipe length (S.L.); (ii) ratio between stipe length (S.L.) and stipe width (S.W.); (iii) ratio between stipe width (S.W.) and bulb width (B.W.). Also, we have used the index of slenderness (I.s. = $l^2/D{\cdot}d$; l = stipe length, D = pileus diameter, d = stipe width) (Kuyper, 1986). All average values of the morphological measurements are represented as underlined values in the TAXONOMY section.

The molecular analysis included 98 ITS sequences in total, from which 17 were generated as part of this study and the remainder taken from GenBank. The selection of the sequences taken from GenBank was based on the morphological analyses carried out by us as well as on the results of Frøslev *et al*. (2007). Thus, we chose 70 sequences of the /calochroid subclade (to which the species studied here belong) and three sequences related to this subclade (one of *C. aureofulvus* M.M. Moser and two of *C. flavovirens* Rob. Henry) (Frøslev *et al*., 2007). *Cortinarius caroviolaceus* P.D. Orton, *C. saporatus* Britzelm., and *C. odorifer* Britzelm. were chosen as outgroup species. EMBL accession numbers for the 17 new sequences are shown in the TAXONOMY part, while the GenBank accession number for the remainder are shown in the ITS tree.

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). 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 98 ITS sequences were aligned using the CLUSTAL option of the MEGALIN program from the DNAstar software package (LASERGEN), followed by a visual inspection. The data matrix is available from TreeBASE (study accession number S1888, matrix accession number M3469). Nine regions (90 characters) were ambiguously aligned in the whole matrix data. However, they were invariable (except for few cases) at intraspecific level and unambiguously aligned among closely related species. Therefore, these regions were considered to calculate the intraspecific distance values and the divergence values between closely related species. In the phylogenetic analysis the ambiguously aligned regions were treated following the method of Lutzoni *et al.* (2000) to account for the phylogenetic information that these regions give. According to this method, first the homologous regions of the alignment containing ambiguously aligned sequences were delimited. Secondly, each of these regions was coded as a new character, replacing its respective ambiguous region. Thirdly, each of the coded characters was subjected to a specific step matrix to account for the differential number of changes needed to transform one sequence into another. We calculated the divergence (*p*-distance) between the sequences using PAUP* version 4.0b10 (Swofford, 2003). Phylogenetic analysis was performed using the maximum-parsimony criterion (MP), as implemented in PAUP* 4.0b10, and it involved heuristic searches. The data matrix was subjected to 1,000 replicates of random sequence additions using tree bisection-reconnection (TBR) branch-swapping under the Fitch criterion (unordered states and equal weights), and MaxTrees setting set to 5,000. Gaps were treated as missing data. Only ten trees were allowed to be held at each step, in order to minimize the time the algorithms spent searching for trees on sub-optimal islands. The starting tree was obtained by stepwise addition. The characters were optimized by accelerated transformation. Finally, 1,000 bootstrap replicates (BS: Felsenstein, 1985) with 10 heuristic searches were performed to assess internal support for nodes. Fig. 1 shows one of the 46 most parsimonious trees obtained from the phylogenetic analysis.

Species identification of the collections studied was based on the analysis of the ITS sequences in combination with the morphological characters. Collections were assigned to a species when their ITS sequences formed a strongly supported monophyletic group with the sequences of that species, with low internal genetic variation and good correlation with the morphological characters. The mean genetic variation (treating the gaps as missing data) within the clades considered as the same species was between 0 and 0.0034 (0-2 nucleotide changes), while this value was between 0.02123 and 0.057 (12-32 changes) for the comparisons between most closely related species according to the tree shown in Fig. 1. Thus, collections with the same ITS sequences or with very low variation between the sequences were considered as the same species.

Table 1 shows the main comparative characters between the studied species, including both morphological, ecological and genetic data.

Taxonomy

Cortinarius haasii var. ***quercus-ilicicola*** A. Ortega, Suár.-Sant. & J.D. Reyes, **var. nov.**

= *Cortinarius haasii* (M.M. Moser) M.M. Moser, Kleine Kryptogamen Flora 2: 294 (1967) *pro parte*.

= *Phlegmacium arquatum* var. *haasii* M.M. Moser, Die Gattung *Phlegmacium*: 353 (1960) *pro parte*.

= *Cortinarius calochrous* var. *haasii* (M.M. Moser) Brandrud in Brandrud & Melot, Nord. J. Bot. 10 (5): 536 (1990) *pro parte*.

= *Cortinarius violaceipes* Bidaud & Consiglio sensu auct., Gal. Hisp. Ital. sensu Atlas des Cortinaires XI: 615 (2001) *pro parte* (AB 92.10.387).

Icon. Sel. : Consiglio *et al.*, Boll. Ass. Micol. ed Ecol. Romana 61, Anno XX (1):31 (2004) (as *C. violaceipes*).

Etymology: The Latin *quercus-ilicicola* refers to the habitat, Mediterranean forest of *Quercus ilex*.

A typo differt carpophoris minoribus, pilei cutis colore pallidiore (flavo vel flavo-ochraceo) et pilei squamulis haud conspicuis. ***Holotypus*** *in Hispania, Granada, parc natural Sierra de Huétor, 1250 m supra mare, solitarius vel gregarius, sub Quercu ilice subsp. ballota, in humo calcarea, 2.XII.2006, lectus, leg. A. Ortega, in herbario GDA sub nº 52537 conservatur.*

Table 1. Main comparative characters between the studied species. Data for KOH macrochemical reaction, index of slenderness (I.s.), spore measurements, plant associates, distribution (Distr.), and genetic distances are shown. Interspecific genetic distances are refered to the most closely related species according to the Fig. 1.

Species	**KOH**		**I.s. (m.v.)**	**Spores (m.v.)**	**Plant associates**	**Distr.**	**Genetic distances**	
	C	B					Intraspecific	Interspecific
C. haasii var. *quercus-ilicicola*	-	-	4.06	11.3-12.2 × 6.7-7.3	*Q. ilex*	M	0.0000 (0.0009 for *C. haasii s.l*)	*C. splendidior*: 0.0212
C. parasuaveolens	+	+	4.71	9.7-11.2 × 5.8-6.8	*Q. ilex*, *Q. coccifera,* *Q. pubescens*	M	0.0018	*C. insignibulbus*: 0.0232; *C. sancti-felicis*: 0.0233
C. platypus	-	-	3.63	10.3 × 5.9-6.1	*Fagus,* *Quercus*	E-M	0.00316	*Cortinarius* sp. AY669563: 0.0422
C. rickenianus	+	+	4.47	9.9-11.4 × 6-6.7	*Carpinus*, *Fagus*, *Quercus*	E-M	0.0000	*Cortinarius* sp. AY669566, DQ323974: 0.02573
C. sancti-felicis	+	+	3.50	10.8-11.2 × 6.6.-6.7	*Q. ilex*	M	0.0005	*C. insignibulbus*: 0.0281
C. selandicus	-	+	7.10	11.3-12 × 6.7-7.4	*Fagus*, *Q. ilex*	E-M	0.0000	*C. calochrous*: 0.0298
C. sodagnitus	+	+	4.95	9.1-10.4 × 5.2-6.2	broadleaf trees	E-M	0.0013	-
C. splendidior	-	-	5.22	11.5-11.9 × 6.8-7.1	*Q. ilex*	M	0.0000	*C. haasii*: 0.0212
C. subgracilis	+	+	5.27	11.8-12.6 × 6.6-7.2	Conifers, *Q. ilex*	E-M	0.0011	*C. chailluzii*: 0.0265

C = cap, B = bulb
\+ = red, - = brown or reddish brown
m.v. = mean values
E = Eurosiberian region, M = Mediterranean region

Pileus 35-60(-70) mm, hemispherical, convex, plano-convex, then plane or slightly depressed at centre, light orange (5A5) or greyish orange (5B4), with whitish yellow or whitish orange (4A3, 5A2), pale yellow (3A3) and greyish yellow (3B3) hues, especially at margin. *Lamellae* crowded, lilac or lilac-violet (16B4, 16C6). *Stipe* (35-)50-60 × 10-17 mm, as long as or smaller than cap diameter (D.P./S.L. = 0.89-1.47) and little or moderately slender (S.L./S.W. = 2.83-5.4), cylindrical, with a broad, flattened marginate bulb (× 22-36 mm); index of slenderness = 1.93-4.06-6.06 greyish violet (17B3) or pale violet (17A3), sometimes only in the upper part. *Basal mycelium* yellowish (4A4-5). *Context* white with violet lilac hues in stipe. *Smell and taste* not distinctive. *KOH* reddish brown on the cap and surface of the bulb (bulbipellis).

Pileipellis an ixocutis, epicutis thick, the upper-layer made up of gelatinized 1.5-3.5 µm wide, erect, ± sinuous, cylindrical hyphae, with slightly clavate terminal cells pigmented with a moderate or strong yellowish or ochraceous-yellowish cellular or encrusted pigment. *The basal part* of the epicutis is formed by loosely interwoven repent hyphae, that, finally, form a thick pseudoparenchymatous lower layer of (sub)parallel, 5-10-13-20 µm wide hyphae with cellular or parietal yellowish pigment. *Basidia* 4-spored, (× 8-10 µm), claviform, hyaline or yellowish. *Marginal cells* rare, (× 7-9 µm) cylindrical or claviform, hyaline. *Spores* (Fig. 2a) 10.8-11.3-12.2-14 × 6-6.7-7.3-8 µm, ellipsoid-amygdaliform, amygdaloid, subcitriform or citriform (Q: L/W: 1.5-1.66-1.72-1.87), with moderate or coarse ± interconnected warts.

Habitat and known distribution: *Cortinarius haasii* is a well-known species in Europe, where it usually grows in conifer woodlands (Brandrud *et al*, 1996; Bidaud *et al.*, 2001).

However, it also grows under broadleaf trees (Frøslev *et al.*, 2007), and it is not rare in the Mediterranean forests of *Quercus ilex*, where also fruits another close taxon: *C. splendidior* Bidaud. Therefore, our data confirm that *C. haasii* and *C. splendidior* share the same habitat in the Mediterranean region.

Material examined: SPAIN, Granada, Huétor Santillán, natural park of the Sierra de Huétor, forest track of the Pajareras, from the forest house of the Peñoncillos to the Fuente de la Teja, Km 0.5, 1250 m, in *Quercus ilex* subsp. *ballota* and *Cistus laurifolius* woodlands, on non-carbonate soil, 2 December 2006, A. Ortega (GDA 52537; **holotype designated here)**, EMBL accession number: AM709874; Jaén, La Iruela, el Cantalar, natural park of the Sierras de Cazorla, Segura y Las Villas, 810 m, under *Quercus ilex* subsp. *ballota*, on calcareous soil, 19 November 2005, J.D. Reyes (GDA 50852); *idem* (GDA 50854) EMBL accession number: AM709873; Siles, la Laguna, Sierra de Segura, 1350 m, same habitat, 17 November 2005, J.D. Reyes (GDA 52538); *idem* (GDA 52539). FRANCE, Murs (Vaucluse), 700 m, sous chênes vertes et buis, in calcaire terrain, 30 October 1992, A. Bidaud (herb. AB 92.10.387 as *C. violaceipes*), EMBL accession number: AM709872.

Notes: The identification of the AB 92.10.387, GDA 50854 and GDA 52537 collections as *C. haasii* was based on morphological and molecular analysis. Fig. 1 shows that their sequences group into a strongly supported clade (97% BS) together the other *C. haasii* sequences. The mean genetic distance value within this clade was very low (p = 0.0009), which together with the morphological evidence supports the species identification of our collections. Conversely to the low intraspecific distance value for *C. haasii*, the interspecific genetic distance value between *C. haasii* and its most closely related species (*C. splendidior*; Fig. 1) was high (p = 0.0212, a mean of 12.42 ITS differences plus 3 indels of one base pair each), which corroborates their specific segregation. On the other hand, a significant datum is the correlation of the intraspecific molecular variation in *C. haasii* with respect to the ecological conditions. The ITS sequences from central European conifer forests populations (AY669561, DQ323966, DQ663311, DQ663312, DQ663315, DQ663316) are grouped together, and they form a sister clade to Mediterranean *Q. ilex*-forests populations (Fig. 1). This differentiation is supported by the values between the sequences, being null the intragroup values (p = 0.0000 both populations under conifers and populations under *Quercus*) while the intergroup mean distance value was p = 0.0017. This latter distance value corresponds to one nucleotide difference, but there is also an indel (of one base pair) differentiating the sequences of both groups. These results support the existence of two ecological variants of *C. haasii* with a different geographical distri-bution, and which we have recognized at the variety level. The Mediterranean sclero-phyllous forests collec-tions of *C. haasii* have some small and stable morphological differ-ences with respect to the central European ones: (i) smaller cap diameter [pileus 35-60(-70) mm *vs*. 50-90 mm], (ii) paler yellowish pileus, which colour is preserved in dried basidiomata, and (iii) absence of ochraceous or cream-coloured scales at disk, which can present whitish veil remnants.

Cortinarius parasuaveolens (Bon & Trescol) Bidaud, Moënne-Locc. & Reumaux, Bull. FAMM 18: 23 (2000).

= *Cortinarius sodagnitus* var. *parasuaveolens* Bon & Trescol, Doc. mycol. XIX (73): 36 (1988).

= *Cortinarius sodagnitus* Rob. Henry *sensu* Brandrud, Breitenbach & Kränzlin, Soop etc. *pro parte*, *non sensu* Frøslev *et al.* (2005).

= *Cortinarius violaceipes* Bidaud & Consiglio, Atlas des Cortinaires XI: 615 (2001). (See Frøslev *et al.*, 2007).

Habitat and known distribution: This species is present in Europe, but it is more frequent in the Mediterranean region, especially in sclerophyllous and mesophilous *Quercus* spp. forests.

Material examined: SPAIN, Álava, Cantonad, in mesophilous *Quercus faginea* and *Q. ilex* forests, 14 November 2004, J.A. Cadiñanos Aguirre (CAD); Córdoba, La Zarcilla, Zuheros, natural park of Sierras subbéticas cordobesas, evergreen oak forest with *Arbutus unedo* and *Viburnum lantana*, 15 November 1992, J. Gómez (GDAC 36403; as *C. sodagnitus*); Zuheros, in *Q. ilex* subsp. *ballota* forests, 850 m, 19 November 2002, J. Gómez *et al.* (JA-Cussta 4868; as *C. sodagnitus*); Girona, coll d'Urra, la Garrotxa, in meso-xerophilous evergreen oak forests, 19 October 1999, J.A. Cadiñanos Aguirre (CAD); Granada, Alhama de Granada, puerto del Navazo, 1200 m, under *Q. ilex* subsp. *ballota*, 10 December 1987, A. Ortega & M.T. Vizoso (GDAC 30809; as *C. sodagnitus*); Cambil, Mata Bejid, evergreen oak forest, 2 December 1987, A. Ortega, M.T. Vizoso & M. Zea (GDAC 30810; as *C. sodagnitus*); *idem*, 27

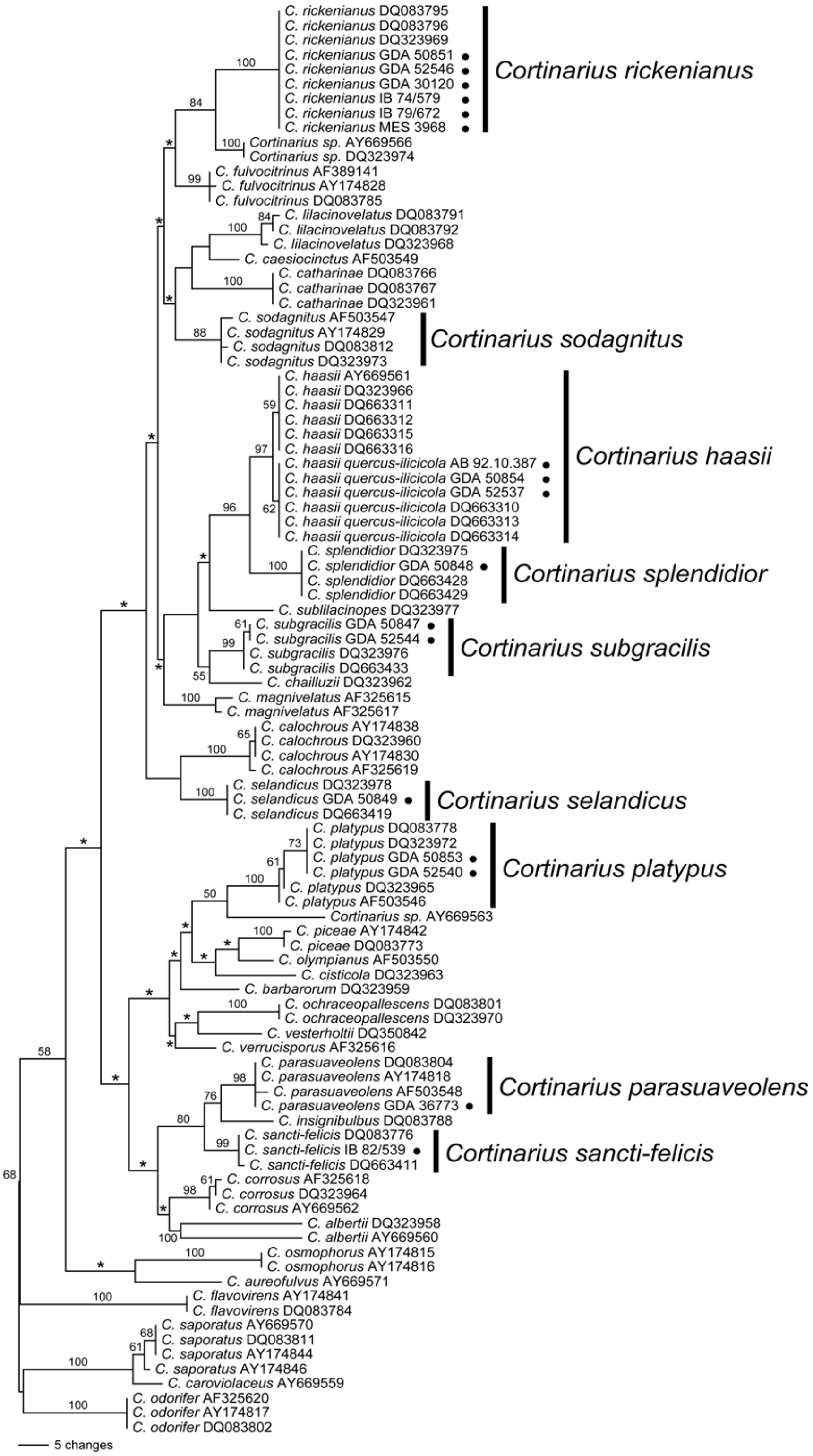

Fig. 1. Phylogenetic relationships of species in the /calochroid subclade sensu Frøslev *et al.*, 2007 (*Cortinarius* sect. *Calochroi* s. auct.). Phylogram of one of the 46 most parsimonious trees (length: 516; CI: 0.593; RI: 0.861; HI: 0.407) obtained from the parsimony analysis. Bootstrap values ≥ 50% are shown above branches. Branches collapsing in the strict consensus tree are marked with an asterisk. 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. Sequences generated in this study are marked with a dark circle. Species studied are indicated at right.

November 1990, A. Ortega & M.T. Vizoso (GDAC 36773; as *C. sodagnitus*), EMBL accession number: AM709875; Cambil, Gibralberca, same habitat, 5 December 2006, J.D. Reyes (GDA 52541, 52542, 52543). FRANCE: Mazet de Romanin, bei St. Rëmy de Provence, under *Q. ilex* and *Q. coccifera*, 20 October 1974, M.M. Moser (IB 74/595); Tour du Cardinal, Alpilles, Provence, same habitat, 24 October 1974, M.M. Moser (IB 74/621).

Notes: In our opinion three characters define this species: (i) the variable colour of the pileus (Cadiñanos-Aguirre, 2004) which discolours intensely when dried, and then the older basidiomata become yellowish-ochraceous or ochraceous; (ii) KOH reddish brown, reddish vinaceous or red on cap, pinkish red on bulbipellis; (iii) Spores: 8.8-9.7-11.2-12 × 5.6-5.8-6.8-7.2 µm. Two species closely related to *C. parasuaveolens* (Fig. 1) are *C. insignibulbus* Bidaud & Moënne-Locc., which has a different habitat (*i.e.* under *Carpinus* and *Fagus*) and smaller spores (Frøslev *et al.*, 2006), and *C. sancti-felicis* Frøslev & T.S. Jeppesen, which no presents or it has lesser presence of violaceous-lilac hues on cap. At the molecular level, the only intraspecific differences for *C. parasuaveolens* were two nucleotide changes between the sequence AF503548 and the remaining *C. parasuaveolens* sequences, while a mean of 13.25 changes (p = 0.0232) plus five indels (one of two base pairs and four of one base pair) and 13.33 changes (p = 0.0233) plus five indels (two of two base pairs and three of one base pair) distinguish the sequences of *C. parasuaveolens* from the those of *C. insignibulbus* and *C. sancti-felicis,* respectively. Sequences of *C. insignibulbus* and *C. sancti-felicis* differ by an average of 16.33 nucleotide changes (p = 0.02813) plus four indels (of one base pair each).

Cortinarius platypus (M.M. Moser) M.M. Moser, Kleine Kryptogamen Flora, 2: 292 (1967).

= *Phlegmacium platypus* M.M. Moser, Die Gattung Phlegmacium: 353 (1960).

Pileus 45-60 mm, convex, plano-convex then plane or, sometimes, slightly depressed at centre. Diversely coloured, GDA 50853: when young pale violet (18A2), pale bluish violet (22A3) or pale purplish grey (9B2, 12B2) mixed with whitish or pale cream hues, after discolouring and then in older basidiomata brownish orange (5B5) or cinnamon brown (8D4); violet (9C3), pinkish vinaceous (12B3), lilac (15B3) universal veil remnants abundant on cap surface, and pale violet (18A2) on bulb; GDA 52540: golden yellow (5B7) and greyish orange (5C3), with several reddish-grey or purplish-grey hues (7B2); whitish or greyish-white universal veil remnants on cap. *Lamellae* crowded, violet (15C4, 15C4) and purplish grey (14A2). Stipe 50-60 × 13-15 mm, slightly slender (S.L./S.W. = 3.8-4.6), cylindrical, with a variable pale violet (19B3) marginate bulb (× 18-35 mm); whitish, with lavender greyish (19A2), lavender (20A3) or pinkish (15A2) diffuse hues, but turning golden yellow (4A7) or greyish orange (5C3) in older basidiomata. *KOH* reddish brown (8D8) in cap and bulbipellis.

Pileipellis an ixocutis, epicutis thick, the upper-layer made up of gelatinized 2-2.5-3(-4) µm wide, erect, ± sinuous, cylindrical, sometimes slightly clavate at top hyphae, hyaline or pigmented, with ± intensely yellowish cellular pigment, some hyphae with encrusted-parietal pigment (older basidiomata). *The basal part* of the epicutis is formed by loosely interwoven repent hyphae, which, finally, form a pseudoparenchymatous layer of (sub)parallel, hyaline, 4-8.5 µm wide hyphae. *Clamps* presents at all septa. *Basidia* 4-spored, (× 7.5-9.5 µm), claviform. *Lamellae* edge with frequent clustered hyaline sterile cells 22-35 × 6.5-16 µm, cylindrical, claviform, sub-pyriform, etc. *Spores* (Fig. 2b) 9.6-10.3-11 × 5.6-5.9-6.1-6.6 µm, amygdaloid or subcitriform [Q: L/W: 1.5-1.67-1.73-1.8(-2)], with moderate or rather coarse ± interconnected warts.

Habitat and known distribution: In the Iberian Peninsula it is known only from Burgos province (northern Spain), growing in *Q. ilex* and *Q. faginea* forests (Cadiñanos-Aguirre, 2004); for this reason our records increase its distribution area. In Europe it fruits under deciduous trees and is not a frequent taxon.

Material examined: SPAIN, Granada, Huétor Santillán, natural park of the Sierra de Huétor, forest track of the Pajareras (from forest house of the Peñoncillos to Fuente de la Teja, Km 0.5), 1250 m *Quercus ilex* subsp. *ballota*, on non-carbonate soil, 9

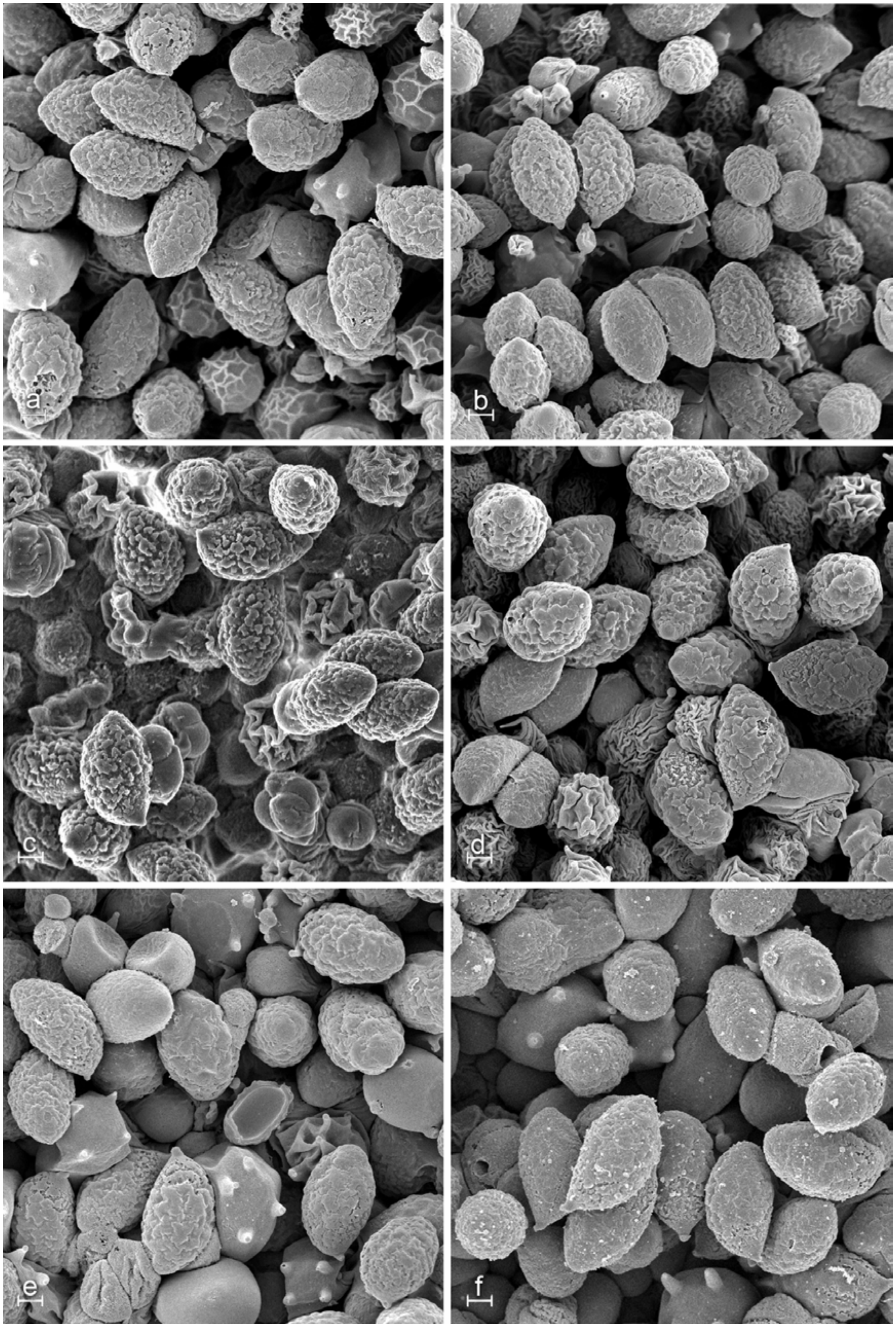

Fig. 2. Field emission scanning electron microscopy (FESEM) of spores. **a.** *Cortinarius haasii* var. *quercus-ilicicola* (holotype). **b.** *C. platypus* (GDA 52540). **c.** *C. rickenianus* (IB 74/579). **d.** *C. rickenianus* (GDA 52546). **e.** *C. splendidior* (GDA 50848). **f.** *C. subgracilis* (GDA 50847). Bars = 2 µm.

December 2006, A. Ortega (GDA 52540), EMBL accession number: AM709877; Jaén, La Iruela, El Cantalar, natural park of the Sierras de Cazorla, Segura y las Villas, 870 m, *Q. ilex* subsp. *ballota*, on calcareous soil, 24 November 2005, J.D. Reyes (GDA 50853), EMBL accession number: AM709876; *idem*, 19 November 2005, (GDA 50855).

Notes: The Andalusian material is morphologically close to that iconographed by Bidaud *et al.* (2001; pl. 526), since their basidiomata have a similar slenderness index (I.s. = 4.26-4.6 *vs*. 3.49-4.36-5.89); however, the iconographic materials by Moser (1960; Fig. 111) and Cadiñanos-Aguirre (2004: 42) show more stocky basidiomata (I.s. = 1.73 and 2.73-2.88, respectively). Moreover, we have observed that the relative bulb width is considerable in the Andalusian collections (S.W./B.W. = 0.3-0.5), except for the collection GDA 52540 (S.W./B.W. = 0.72), and this character is observable also in the above-mentioned iconographic materials. Similarly, several differences in the cap and bulb colour distinguish the two Andalusian populations. Nevertheless, their spore size and morphology of sterile edge cells are similar. ITS sequences completely resolve the taxonomic ascription of both Andalusian collections, since their sequences come into the *C. platypus* clade (Fig. 1), and they coincide entirely with the sequence of the type material (DQ083778; $p = 0.000$). Frøslev *et al.* (2007) demonstrated that *C. platypus* is a genetically polymorphic species, since they found six polymorphic sites in the ITS sequences of this species, three of which were also seen as intragenomic polymorphisms; and they identified two allelic ITS variants ("a", to which the sequences of our collections belong, and "b", represented by the sequences AF503546 and DQ323965). However, as these authors noted and we have checked in our collections, the intraspecific molecular variation in *C. platypus* is not correlated with any other character (morphological or ecological). Therefore, all the aforementioned evidence does not distinguish any infraspecific taxa in *C. platypus*.

Cortinarius rickenianus Maire, Fungi Catalaunici II: 111 (1937).

= *Cortinarius nymphicolor* Reumaux, Atlas des Cortinaires V: 151 (1993).

= *Cortinarius rickenianus* Maire *sensu* M.M. Moser, herb. IB n. 74/579, 79/672.

= *Cortinarius molochinus* Bidaud & Ramm in Bidaud *et al*., Atlas des Cortinaires XI (2): 613 (2001).

= *Cortinarius rickenianus* Maire *sensu* Ramm & Henry, Bull. Soc. Mycol. France 109: pl. 266 (1993).

= *Cortinarius* cf. *molochinus* Ramm, Doc. Mycol. XXV (97): 65 (1995).

Pileus 37-90 mm, convex, trapezoidal-convex, then plane or sometimes concave-depressed at centre: when young, pale violet (19A2) and pale bluish (20A3), soon discolouring and then golden yellow (5B7), orange (5B8), yellowish orange (4A7) or yellowish (3A3,5, 4A3), older basidiomata pale yellowish. Whitish violet-blue (17A2, 18A2) and purplish whitish lilac (14A2) fibrillose *universal veil remnants* abundant on cap surface. *Lamellae* crowded, pale violet (17B4) and mauve or pale lilac (16B4), these tinges persisting even in the dried herbaria material. *Stipe* 50-150 × 10-20 mm, slender (S.L./S.W. = 3.8-7.7), cylindrical, with a marginate bulb (× 20-35 mm); whitish, with a bluish lilac tinge (19A3) under the lamellae, but turning golden yellow (4A7) or yellowish (4A3) in older basidiomata. *Context* white, bluish lilac-violet (19A3) at the top of the stipe and yellow-ochraceous at the bulb. *Smell and taste* not distinctive (these characteristic refer to the material from Granada, Spain). *KOH* reddish pink (12 A-6) in cap and in the bulbipellis.

Pileipellis an ixocutis, epicutis thick, made up of an upper layer of gelatinized, 2-2.5-3(-4) µm wide, erect, ± sinuous, cylindrical, sometimes with a slightly clavate top hyphae, hyaline or pigmented, with ± intensely yellowish cellular pigment. *The basal part* of the epicutis is formed by loosely interwoven repent hyphae, which finally form a pseudoparenchymatous layer of (sub) parallel, hyaline, hyphae 5-8 µm wide. *Clamps* presents at all septa. *Basidia* 4-spored, (× 8-9.5 µm), claviform. *Sterile cells* 25-35 × 8-12 µm, hyaline, cylindrical, claviform, subpyriform, etc. Spores (Figs. 2c,d) 9.2-9.9-11.4-12.2 × 5.6-6-6.7-7.2 µm, amygdaloid or subcitriform (Q: L/W: 1.5-1.60-1.73-1.9), with moderate or rather coarse ± interconnected warts.

Habitat and known distribution: This species has been collected from several French localities (Bidaud *et al.*, 2001), also from

Czech Republic and Switzerland (Frøslev *et al.*, 2005), and Slovenia (IB 79/672). For this reason our record substantially increases its distribution area. This species is frequent in the Mediterranean area.

Material examined: SPAIN, Castelló, Tinença de Benifassá, 650 m, in *Quercus ilex* subsp. *ballota* and *Pinus halepensis* forests, on calcareous soil, 1 November 2003, R. Mahiques (MES 3968; as *C. parasuaveolens*), EMBL accession number: AM709883; Granada, Huétor Santillán, natural park of the Sierra de Huétor, forest track of the pajareras (from forest house of the Peñoncillos to Fuente de la Teja, Km 0.5), 1250 m, *Q. ilex* subsp. *ballota*, on non-carbonate soil, 10 December 2005, A. Ortega (GDA 50851), EMBL accession number: AM709878; *idem*, 2 December 2006, A. Ortega (GDA 52545); Güéjar Sierra, near to Hotel Santa Cruz, 1450 m, under *Q. ilex* subsp. *ballota*, on non-carbonate soil, 1 November 1989, Ortega *et al.* (GDAC 30120; as *C. sodagnitus* var. *parasuaveolens*), EMBL accession number: AM709880; Jaén, Cambil, Gibralberca, under *Q. ilex* subsp. *ballota*, 6 December 2006, J.D.Reyes (GDA 52546), EMBL accession number: AM709879; *idem*, (GDA 52547, 52548, 52549). FRANCE, Mazet de Romanin, St. Rémy de Provence, in *Quercetum* (*Q. ilex* and *Q. coccifera*), 20 October 1974, M.M. Moser (IB 74/579; as *C. rickenianus*), EMBL accession number: AM709881. SLOVENIA, Grcevec, ca 40 Km Südöstl. Laibach, mistewald (Büche, *Carpinus*, Eiche, Fich), 10 October 1979, M.M. Moser (IB 79/672; as *C. rickenianus*), EMBL accession number: AM709882.

Notes: The Andalusian material corresponds strictly to the morphological concept of *C. molochinus* (= *C. rickenianus sensu* Ramm & Henry), and it is very close to French mycologists' concept of *C. nymphicolor* (= *C. rickenianus sensu auct.*) and to *C. parasuaveolens* (= *C. sodagnitus sensu lato, pro parte*). Also it is related to some members of the *C. parvus* group (*e.g. C. lilacinovelatus* Reumaux & Ramm) according to the data published by Frøslev *et al.* (2005).

In the opinion of French mycologists the presence of pinkish-lilac or violaceous hues on the cap surface and the pinkish-red KOH reaction relate *C. molochinus sensu str.* to *C. nymphicolor sensu auct. galliae*, *C. parasuaveolens*, and *C. sodagnitus*; all these species also fruit in Mediterranean *Quercus* forests.

Cortinarius rickenianus sensu lato differs from *C. parasuaveolens* by its paler pileus with pinkish-lilac hues, while *C. parasuaveolens* has a more yellowish or yellowish-ochraceous pileus with lilac-violaceous or violet hues. Moreover, the *C. rickenianus* stipe is whitish except at the apex, while in *C. parasuaveolens* it is yellowish with violaceous or lilac hues. The KOH reaction is pinkish-red on the cap and bulbipellis in *C. rickenianus*, and of the same colour (but inconstant on pileus) in *C. parasuaveolens*. Bidaud *et al.* (1993; f. 70) suggested that the pileipellis structure differs slightly between the two species, *C. parasuaveolens* having a wider and more compact pseudoparenchymatous basal layer. This character was not observed by us.

We analysed the ITS sequences of two collections of *C. rickenianus sensu* M.M. Moser (= *C. nymphicolor* Reumaux) from IB (IB 74/579; IB 79/672) which agree completely (p = 0.000; Fig. 1) with the three sequences considered by Frøslev *et al.* (2006, 2007) as *C. nymphicolor* (DQ083795, DQ 083796, DQ323969), one of which coming from the type material of *C. molochinus*. For this reason, we believe that *C. rickenianus*, *C. nymphicolor* and *C. molochinus* correspond to the same species. In terms of nomenclature, Frøslev *et al.* (2006, 2007) considered *C. molochinus* to be synonymous with *C. nymphicolor* because the latter name was older; however, according to our results, *C. rickenianus* Maire has priority.

The southern Spain collections respond to two biotypes of *C. rickenianus*. One (GDA 50851, GDA 52545, GDA 52546, GDA 52549) has slender basidiomata [I.s. = 5.6-$\underline{7.05}$-8.3(-12.3)], while the other (GDAC 30120, GDA 52547, GDA 52548) has stocky basidiomata (I.s. = 2.2-$\underline{2.8}$-3.5). These latter coincide with the traditional concept of this species [I.s. = 1.8-$\underline{3.2}$-6(-8)] (Bidaud *et al.*, 1993, 2001; Cadiñanos-Aguirre, 1995; Soop, 2005, etc.). Moreover, we noted clear differences in the spore size within the Andalusian material, as some collections have larger mean values (11.4-12.8 × 6-6.8 µm) and others have smaller mean values (10-10.8 × 6-6.4 µm). However, slenderness and spore size did not correlate. These results show that *C. rickenianus* is a highly variable species, in which the morphological variability of the basidiomata and spore size correspond to the phenotypical plasticity of this species, since the spore dimensions could differ according to environmental parameters (e.g. temperature flucutations; Clemençon, 1997).

Cortinarius lilacinovelatus is relatively close to *C. rickenianus* in terms of morphology, but differs markedly in the typical olivaceous to slightly greenish yellow hues on the cap (lacking in *C. rickenianus*), and the different KOH reaction on the cap and bulbipellis (Bidaud *et al*., 2001, Consiglio *et al*., 2004, Frøslev *et al*,. 2006). From a molecular standpoint, the ITS sequences of the two species are substantially different (mean distance p = 0.04888, 28.33 nucleotide changes in average), and they show that they are phylogenetically distant (Fig. 1).

Cortinarius sancti-felicis Frøslev & T.S. Jeppesen, Mycol. Res. 110: 1148 (2006).

= *Cortinarius rickenianus* Maire *sensu* M.M. Moser *et al*., Colour Atlas of Basidiomycetes III: 13 (1986).

Pileus 40-80 mm, hemispherical, convex, plano-convex, plane or slightly depressed at centre, with lubricous and shining surface, when younger pale orange (5A3) with a slight lilac hue (10A2) and then ochraceous orange (6B4-6) or reddish brown (7-8C8), smooth when younger, the older basidiomata with innate ± radial fibrils and little darker scales at disk, so that the cap appears to be bicoloured, lilac-violaceous (13-14B4) universal veil remnants scarce and little evident. *Stipe* 40-70 × 12-18 mm, cylindrical, with a rounded marginate bulb (× 30-40 mm), lilac-violet (12B4-5), in older specimens turning brownish, bulb lilac-violet (13-14B4) when younger, whitish or yellowish (4A3) in adults. *Lamellae* lilac-violet (12B4). *Context* white in the pileus and lilac (12B3) in the stipe. (These characteristics refer to the material represented in Moser *et al*., 1986; *Cortinarius* 13). *KOH* red in bulbipellis (12A6-7-8) and cap (13A8) (especially on younger caps).

Pileipellis an ixocutis, consisting of a thick epicutis, with gelatinized thin hyphae (× 1.5-4 µm), erect, with numerous cylindrical free ending cells, and a lower-layer formed by loosely interwoven or ± parallel repent yellowish, 4-7 µm wide hyphae, with pigment intracellular (some hyphae with encrusted-parietal pigment). *Spores* 10.4-$\underline{10.8\text{-}11.1}$-12 × 6.2-$\underline{6.6\text{-}6.7}$-7.2 µm, ellipsoid, amygdaliform, subcitriform or citriform (Q: L/W = 1.5-$\underline{1.61\text{-}1.69}$-1.9), ochraceous yellowish (O.M), with coarse ± interconnected warts.

Habitat and known distribution: This species has been collected only twice from southern France and Catalonia (Spain) (Frøslev *et al*., 2006) under *Quercus ilex*. For this reason we believe that *C. sancti-felicis* could be a taxon of Mediterranean distribution, perhaps confused with *C. rickenianus* in other European zones.

Material examined: FRANCE, Tour du Cardinal après St Remy, B. du Rhône, under *Quercus ilex*, 2 November 1982, M.M. Moser (IB 82/539; as *Cortinarius* aff. *rickenianus*), EMBL accession number: AM709884.

Notes: The material studied from IB was identified as *C.* aff *rickenianus* by Moser. This signifies that this author noted some differences with respect to the typical *C. rickenianus*, as a lesser presence of pinkish-lilac-violaceous hues on cap.

The basidiomata of the IB sample (Moser *et al*., 1986) look very similar to the collections studied by Frøslev *et al*. (2006) (P.D./S.L. = 1.09-1.27; S.L./S.W. = 1.67-5.4; S.W./B.W. = 0.35-0.5 *vs***.** P.D./S.L. = 1-1.33; S.L./S.W. = 2.5; S.W./B.W. = 0.36-0.5; I.s. = 1.5-4.9 *vs*. 2.6-4.1), but they present several differences, perhaps because of a peculiar species variability: (i) the Danish mycologists affirm that *C. sancti-felicis* has olive-greenish hues on cap and has little brown-reddish scales on the disk; these characters are lacking in IB 82/539; (ii) there are violet-lilac (13-14B4) universal veil remnants on the cap and the bulb border in IB 82/539, while according to Frøslev *et al*. (*loc. cit.*) the veil remnants are less evident and less coloured; (iii) the KOH red reaction on the cap was not observed by Danish mycologists, except for the universal veil remnants on pileus, while in IB 82/539 this reaction is positive.

Despite these morphological divergences, the molecular data strongly support the identity of the collection IB 82/539 as *C. sancti-felicis*. Fig. 1 shows a strong grouping (BS: 99%) between the ITS sequences of this collection and the other collections (including the type material); moreover, the similarity between the sequences of the type material and our collection is 100%.

Cortinarius selandicus Frøslev & T.S. Jeppesen, Mycol. Res. 110: 1148 (2006).

Pileus 55-65(-70) mm diam., hemispherical, convex, parabolic, plano-convex, then plane or sometimes slightly depressed at the centre. *When young*, bluish white (21A2), very pale bluish (19A3) or pale rose lilac (18A2), then quickly yellowing, and then orange (6C8) with greyish yellow hues (3B5, 4B5), without any lilac or violet hues in older basidiomata. *Universal* lilac (15B4) veil present on cap surface and bulb: in the older specimens the fibrillose veil remnants are whitish and sometimes, with age, innately fibrillose. *Lamellae* crowded, lilac or lilac-violet (13B3). *Stipe* 60-70 × 8-10 mm, as long as cap diameter (D.P./S.L. = 0.92-0.96) and slender (S.L/S.W. = 7-7.8), cylindrical, with a flattened-marginate bulb (× 17-25 mm); index of slenderness = 7.31-8.18; violet or lilac-violet (17A4), sometimes turning ochraceous-orange (6B8, 6C8), only at the apex, in older basidiomata. *Bulb* white or ochraceous yellowish, basal mycelium whitish. *Context* lilac, then white in stipe, yellowish, then greyish orange at the cap and bulb. *Smell and taste* not distinctive. *KOH* reddish brown on the cap, red on bulbipellis.

Pileipellis an ixocutis, epicutis thick, made up of a gelatinized upper layer of 2.5-4 µm wide, erect, ± sinuous, cylindrical hyphae, with a slightly clavate or contorted top, hyaline or more frequently pigmented, with a yellowish cellular or parietal pigment. In older basidiomata with yellow-ochraceous cap, these hyphae have a strongly encrusted pigment. *The basal part* of the epicutis is formed by loosely interwoven repent hyphae, which, finally, form a thick pseudoparenchymatous lower layer of (sub)parallel, 4-10-16 µm wide hyphae with cellular or parietal yellowish pigment. *Basidia* 4-spored, (× 8-9.5 µm), claviform, hyaline or yellowish. *Spores* 10.2-11.3-11.6-12-13 × 6.2-6.7-7.2-7.4-8.4 µm, ellipsoid-amygdaliform, amygdaloid, subcitriform or citriform (Q: L/W: 1.47-1.61-1.63-1.69-2), with moderate or coarse ± interconnected warts.

Habitat and known distribution: This species had previously been collected only three times, in *Fagus sylvatica* forests in Denmark (Frøslev *et al.*, 2006). Therefore, our record under *Quercus ilex* subsp. *ballota*, significantly increases its distribution area and its ecological and biogeographical range.

Material examined: SPAIN, Granada, Huétor Santillán, natural park of the Sierra de Huétor, forest track of the Pajareras (from forest house of the Peñoncillos to Fuente de la Teja, Km 0.5), 1250 m, under *Quercus ilex* subsp. *ballota*, on non-carbonate soil, 31 December 2005, A. Ortega (GDA 50849), EMBL accession number: AM709885.

Notes: We would like to point out the pronounced differences in the spore dimensions between our samples (mean values 11.3-12 × 6.7-7.4 µm) and the Danish collections (mean values 9.7 ± 0.4 × 6 ± 0.4 µm), although they have a similar shape [Q: L/W = 1.61-1.69 *vs.* Q: L/W = 1.62 ± 0.08 (Frøslev *et al.*, 2006)]. However, the spore-size variability disagrees with the high degree of similarity (100%) of their ITS sequences. This supports the idea that spore size has limited taxonomic value in this group under the phylogenetic species concept (as indicated previously).

Cortinarius sodagnitus Rob. Henry, Bull. Soc. mycol. Fr. 51: 44 (1935).

Habitat and known distribution: This species can be collected from many zones of Europe, although it is more present in the northern regions, where is a frequent taxon in hydrophilous and mesophilous deciduous forests.

Material examined: SPAIN, Navarra, Acedo, under *Quercus ilex*, 19 November 1988, L.M. García Bona (MN 3378). GERMANY, Westl. Karlburg, Karlstadt, Bayern, Buchenwald auf Muschelkalk, 25 September 1980, M.M. Moser (IB 80/408). ITALY, Prov. L'Aquila, comune di Opi, Abruzzo, parco naturale d'Abruzzo, Val Fondillo, under *Fagus sylvatica*, 25 September 1999, M.M. Moser (IB 1999/0209); *idem*, 1150-1250 m, under *F. sylvatica* with *Picea*, *Larix*, *Juniperus*, on calcareous soil, 19 September 2001, GEMA (IB 2001/0731); prov. di Grosseto, near Mte. S. croce, strasse 439 nach Larderello, under *Q. cerris*, 26.10.1992, M.M. Moser (IB 92/335).

Notes: From our perspective, three characters define this species: (i) the cap soon discolouring and then taking on a cream-yellowish or yellowish colour, (ii) KOH red on cap and bulbipellis, (iii) spores: 8.6-9.1-10.4-11.2 × 4.6-5.2-6.2-6.8 µm. From a morphological point of view, *C. parasuaveolens* could be a close species to *C. sodagnitus*, which grows in more meridional and dried ecosystems. However, both taxa can be distinguished by the following features: (i) younger cap violaceous-lilac and uniform coloured in *C. sodagnitus*, that soon discolours to cream-yellowish or yellowish colour, while *C. parasuaveolens* has a less clearly defined colour on the cap, that discolours to yellowish-ochraceous or ochraceous. (ii) spores 9.1-10.4 × 5.2-6.2 µm (mean values) in *C. sodagnitus*; 9.7-11.2 × 5.8-6.8 µm (mean values) in *C. parasuaveolens*. At the molecular level, both species can be easily distinguished, since the mean distance value between the ITS sequences of both species is high ($p = 0.03135$; 16.8 nucleotide changes in average).

Cortinarius splendidior Bidaud, Atlas des Cortinaires XI (2): 614 (2001).

Pileus 35-50(-55) mm diam., hemispherical, convex, plano-convex, then plane or, in older specimens slightly depressed at centre, with a diverse colour in adult basidiomata: topaz yellow (5C5), Pompeian yellow (5C6), light brown (5D7), golden brown (5D8) and greyish brown (11D3), with dull yellow (3B3), chamois (4C5) and pale green (30A3) hues at margin, when young smooth, then with some darker radial innate fibrils; whitish universal veil remnants present in several specimens. *Lamellae* crowded, lilac or lilac-violet (16B3-4). *Stipe* 45-55 × 10-12 mm, as long as or larger than cap diameter (D.P./S.L. = 0.78-1) and moderately slender (S.L/S.W. = 4-5), cylindrical, with a flattened or rounded marginate bulb (× 18-25 mm); index of slenderness = 4.58-<u>5.22</u>-6.35; violet (17B3) or pale violet (17A3), sometimes, only at apex, turning yellowish ochre (5C7) or ochre orange (6C8) in older basidiomata. *Bulb* white or yellowish, violet-lilac (16B5) at border; cortina abundant violet-lilac, basal mycelium yellowish (4A4-5). *Context* white with light yellowish (4A3) hues in cap, violet lilac in stipe, yellowish (4A4) at bulb. *Smell and taste* not distinctive. *KOH* reddish brown on cap and bulbipellis.

Pileipellis an ixocutis, epicutis thick, made up of a gelatinized upper-layer of 2.5-4 µm wide, erect, ± sinuous, cylindrical, with slightly clavate or contorted top, hyphae, pigmented with a yellowish cellular or parietal pigment. *The basal part* of epicutis is formed by loosely interwoven repent hyphae, which finally form a thick pseudoparenchymatous lower layer of (sub)parallel, (× 4-10-16 µm) hyphae with cellular or parietal yellowish pigment. *Basidia* 4-spored, (× 8-10 µm), claviform, hyaline or yellowish. *Sterile cells* frequent, [× 7-9(-15) µm], hyaline, cylindrical, claviform, sublageniform or pyriform. *Spores* (Fig. 2e) 10.4-<u>11.5-11.7-11.9</u>-13 × 6.4-<u>6.8-7-7.1</u>-7.4 µm, ellipsoid-amygdaliform, amygdaloid, subcitriform or citriform (Q: L/W: 1.5-<u>1.65-1.69-1.71</u>-1.87), with moderate or coarse ± interconnected warts.

Habitat and known distribution: This species grows under *Quercus ilex*, and its distribution area is typically Mediterranean. At the moment it is well-known only from southern France (Bidaud *et al*., 2001; Frøslev *et al*., 2007) and from Catalonia (Frøslev *et al*., 2007). Therefore, our records increase its distribution area.

Material examined: SPAIN, Granada, Huétor Santillán, natural park of the Sierra de Huétor, forest track of the Pajareras (from forest house of the Peñoncillos to Fuente de la Teja, Km 0.3), 1250 m, under *Quercus ilex* subsp. *ballota*, on calcareous soil, 15 January 2006, A. Ortega (GDA 50848), EMBL accession number: AM709886; *idem*, 31 December 2005 (GDA 50850); *idem*, forest track of the Pajareras (from forest house of the Peñoncillos to Fuente de la Teja, Km 0.5), 5 January 2007 (GDA 52550).

Notes: This species is morphologically and phylogenetically very close to *C. haasii sensu lato*. The habitat in which *C. splendidior* grows (*Quercus ilex* woodlands in Mediterranean areas) distinguishes this species from *C. haasii* var. *haasii* (growing in conifers woodlands in the Eurosiberian region). However, the separation from *C. haasii* var. *quecus-ilicicola* is more difficult, since both taxa share the same habitat. *Cortinarius splendidior* can be morphologically distinguished from *C. haasii* var. *quercus-ilicicola*: (i) basidiomata, on average, smaller (pileus 35-50(-55) mm diam. and stipe 45-55 × 10-12 mm *vs* pileus 35-60(-70) mm and stipe 50-60 × 10-17 mm), and more slender (Index slenderness = 5.22 *vs* 4.06); (ii) pileus ochraceous with olivaceous hues *vs* pileus yellowish or yellowish orange. As we reported in the *Notes* for *C. haasii*, the ITS sequences corroborate the specific segregation of both species.

Cortinarius subgracilis Moënne-Locc., Atlas des Cortinaires XI (2): 614 (2001).

Pileus 32-40 mm (in GDA 52544) and 30-60 mm in (GDA 50847), hemispherical, convex, plano-convex, then plane or, in older specimens, slightly depressed at centre, ivory (4B3) with greyish orange (6B3), lilac or violet hues (in GDA 52544); yellow (4B8) with golden yellowish (5B8) and brownish yellow (5C8), whitish-violet (16A2) or pale violet (16A3) hues (in GDA 50847). *When young* smooth, then with some darker innate radial fibrils, whitish universal veil remnants present in several specimens. *Lamellae* crowded, pink (12A4), pale violet (17A3), light violet (18A4) or greyish violet (17B3). Stipe 35-50 × 8-12 mm, long as or smaller than cap diameter (D.P./S.L. = 0.75-1.2) and moderately slender (S.L/S.W. = 4.2-5.4), index of slenderness = 3.12-5.27-7.14, cylindrical, with a flattened and irregularly marginate bulb (× 15-20 mm); pale violet (16A3) or greyish violet (17B3-5), sometimes, turning whitish, greyish-orange (6B3) or brownish-orange (6C8) at apex in older basidiomata. *Bulb* white or yellowish, sometimes violet-lilac (16B5) at border; cortina abundant, basal mycelium whitish. *Context* white in cap, greyish-orange (5B3) in the base of stipe and in bulb. *Smell and taste* not distinctive. *KOH* red on the cap and bulbipellis.

Pileipellis an ixocutis, epicutis thick, made up of a gelatinized upper-layer of very long, erect, ± sinuous, cylindrical, 2-4 µm wide hyphae with a slightly clavate top, pigmented with a yellowish cellular or parietal pigment. *The basal part* of the epicutis is formed by loosely interwoven repent hyphae, which finally form a thick pseudoparenchymatous lower layer of (sub)parallel, 5-10-15 µm wide hyphae with cellular or parietal yellowish pigment. *Basidia* 4-spored, (× 7.5-9.5 µm), claviform, hyaline or yellowish. *Spores* (Fig. 2f) 10.4-11.8-12.3-12.6-14 × 6-6.6-6.8-7.2-8.4 µm, sub-cylindrical, ellipsoid-amygdaliform, amygdaloid or subcitriform (Q: L/W: 1.5-1.71-1.74-1.79-1.83-2.1), with moderate ± interconnected warts.

Habitat and known distribution: According to the literature this species grows in conifer forests under *Picea* spp., and is known only from France (Bidaud *et al.*, 2001) and Sweden (Frøslev *et al.*, 2007). Our records from the south of the Iberian Peninsula significantly increase its distribution area. Another important datum refers to the Mediterranean habitat of the Spanish records, where our collections grow in *Quercus ilex* subsp. *ballota* woodlands, this being a very different habitat with respect to *Picea* spp. forests. For these reasons, we believe that *C. subgracilis* could have a greater ecological range and wider geographical distribution in Europe.

Material examined: SPAIN, Granada, Huétor Santillán, natural park of the Sierra de Huétor, forest track of the Pajareras (from forest house of the Peñoncillos to Fuente de la Teja, Km 0.3), 1250 m, under *Quercus ilex* subsp. *ballota*, on calcareous soil, 15 January 2006, A. Ortega (GDA 50847), EMBL accession number: AM709887; Jaén, Siles, la Laguna, Sierra de Segura, same habitat, 13 November 2005, J.D. Reyes (GDA 52544), EMBL accession number: AM709888.

Notes: Fig. 1 shows the ITS sequences of the two Spanish collections grouping strongly (BS: 99%) to the other *C. subgracilis* sequences, including the type-material sequence, since only one nucleotide position distinguishes the sequences of the Mediterranean collections from the Eurosiberian ones. However, the Spanish collections show evident morphological differences with respect to the original description of this taxon (Bidaud *et al.*, 2001), as well as between themselves. Thus, the GDA 50847 collection has larger basidiomata (cap: as much as 60 mm diam.) and the pileus has pale violet hues; while these characteristics are not present either in the GDA 52544 collection or in the original material described by Moënne-Loccoz (Bidaud *et al.*, *loc. cit.*). Therefore, the remarkable morphological variability found in *C. subgracilis* disagrees with its genetic homogeneity, particularly for the Mediterranean quercicolous collections (p = 0.000), and therefore it could be attributed to the morphological variability of this species (which is poorly known). The current data do not support the differentiation of two variants (the one from southern holm-oak forests and the other from northern *Picea* spp. forests). More data from Mediterranean and central-European material are necessary to determine whether the differences detected are stable throughout populations of each region and ecosystem.

Key to the species of *Cortinarius* studied

1. KOH red on the cap and/or bulbipellis 2
1' KOH reddish brown on the cap and bulbipellis..........7

2. KOH red on the bulbipellis only........... ***C. selandicus***
2' KOH red on the cap and bulbipellis............................3

3. Spores on average longer than 11.5 µm.......................
....***C. subgracilis***
3' Spores on average shorter than 11.5 µm.....................4

4. With violaceous colour on the younger cap 5
4' With pale lilac or lilac pinkish on the younger cap .....
....6

5. Colour of the younger pileus variable, older basidiomata become ochraceous. In sclerophyllous woodlands .... ***C. parasuaveolens***
5' Colour of the younger pileus uniformly violaceous, older basidiomata become cream-yellowish or yellowish. In mesophilous and hydrophilous woodlands..................
....***C. sodagnitus***

6. Evident pale lilac or lilac pinkish hues on the younger cap .... ***C. rickenianus***
6' Hardly observable pale-lilac or pinkish-lilac hues on the younger cap.......................................***C. sancti-felicis***

7. Mycelia strands not yellow .... ***C. platypus***
7' Mycelia strands yellow..8

8. Taxa growing in conifer woodlands ....
....***C. haasii* var. *haasii***
8' Taxa growing in *Quercus ilex* woodlands 9

9. Pileus 35-60(-70) mm diam., slenderness index on average = 4.06; cap without olivaceous hues ....
......................................***C. haasii* var. *quercus-ilicicola***
9' Pileus 35-50(-55) mm diam., slenderness index on average = 5.22; cap with olivaceous hues ....
.... ***C. splendidior***

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