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## PRELIMINARY MORPHOLOGIC AND MOLECULAR STUDY OF THE *ENTOLOMA RUSTICOIDES* GROUP (AGARICALES - BASIDIOMYCOTA)

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**SUMMARY.** A study of the *Entoloma rusticoides*-group (subgenus *Claudopus*) in the Iberian peninsula is presented. Phylogenetic inference, based on 48 nrITS and 45 28S nrLSU sequences, including those of 8 type specimens, revealed a high genetic diversity, in accordance with the observed morphological variability in this group. In addition new taxa are proposed to accommodate independent lineages (*E. almeriense*, *E. graphitipes* f. *cystidiatum*, *E. halophilum*, *E. muscoalpinum* and *E. phaeocarpum*) and the typification of *E. phaeocyathus* and *E. rusticoides* is proposed. Preliminary taxonomic keys are included to help identification. For most species macro and microscopic descriptions, drawings and photographs are presented.

**KEY WORDS:** *Entolomataceae*, *Entoloma*, *Claudopus*, taxonomy, ITS-LSU phylogeny, Iberian peninsula.

**RESUM.** Es presenta un estudi sobre el grup *rusticoides* del gènere *Entoloma* subgènere *Claudopus* a la Península Ibèrica. L'anàlisi filogenètica, sobre la base de 48 seqüències d'ITS i 45 seqüències de LSU, entre les quals, les dels tipus de 8 dels tàxons analitzats, mostra una marcada diversitat genètica que concorda amb la notable variabilitat observada en aquest grup. D'acord amb aquest fet, es descriuen els següents nous tàxons: *E. almeriense*, *E. graphitipes* f. *cystidiatum*, *E. halophilum*, *E. muscoalpinum* i *E. phaeocarpum*. A més, es tipifiquen *E. phaeocyathus* i *E. rusticoides*. S'inclouen claus provisionals d'identificació. S'aporten descripcions macro i microscòpiques, dibuixos i fotografies de la majoria dels tàxons estudiats.

**PARAULES CLAU:** *Entolomataceae*, *Entoloma*, *Claudopus*, taxonomia, filogènia molecular, marcadors ITS-LSU, Península Ibèrica.

### INTRODUCTION

The large number of reviews to which the genus *Entoloma* has been subjected demonstrates its taxonomic difficulty and complexity (see NOORDELOOS, 1992, 2004; LUDWIG, 2007; VILA & CABALLERO, 2007, 2009; CO-DAVID et al., 2009; CABALLERO & VILA, 2013; HE et al., 2013). Systematics in this genus has traditionally been based on macro and microscopic features. This has led, however, to much confusion in identification (see VILA et al., 2013). Phylogenetic studies have revealed that some characters are inadequate to correctly identify these species, since they are too variable (VILA et al., 2013; MORGADO et al., 2013; MORZOVA et al., 2014).

For example, within subgenus *Claudopus*, section *Undata* (NOORDELOOS, 2004), the species related to *Entoloma rusticoides* appear as monophyletic, both in molecular and morphological studies. Macroscopically, most taxa have (i) small-sized basidiomata (the pileus rarely exceeds 20 mm in diam.), rather dark in colour, ranging from brown to grayish-brown, grey or even blackish, (ii) a short stipe, no longer than the pileus diameter, or even shorter, and (iii) distant and thick lamellae. Microscopically, they have (iv) clampless hyphae, (v) a dominant incrustant epiparietal pigment (sometimes mixed with intraparietal or vacuolar pigment), and (vi) large spores, iso- or subisodiametrical, with weak angles often giving a rounded appearance to the perimeter. These traits allow an easy discrimination from the remaining lineages of section *Undata*, which commonly show larger basidiomata, differing in colour, a stipe longer than the diameter of the pileus, heterodiametrical spores, and clamped hyphae. Section *Claudopus*, which includes species with a reduced or even absent stipe, seems more closely related to the lineage of *E. undatum* than to that of *E. rusticoides*.

## MATERIAL AND METHODS

### A) HERBARIUM MATERIAL

Material was obtained from the herbaria K (Kew, England), M (Munich, Germany), and the personal herbaria of A. Caballero (AC), J.F. Vicente (FDEZ), R. Mahiques (MES), and A. Conca (ACM). In addition, we examined the private collections of the authors: JC (J. Carbó), SCG (S. Català), EFC-SFC (F. Caballero), JVG (J. Vila), and MHR (M.A. Higuelmo). The holotypes of the newly described taxa, as well as the epitypes, are deposited in LIP (Lille, France). Since numerous taxa treated in this article were described in the work of LUDWIG (2007), we asked on loan several collections to Munich (M), where most of E. Ludwig's collections are kept. Unfortunately, we could only obtain a few sequences from these samples, probably because of their poor condition - often moldy or damaged.

### B) DNA EXTRACTION AND SEQUENCING

The DNA was extracted from 15-30 mg of dry material (depending on availability), using E.Z.N.A.® Plant DNA Kit (Omega Bio-Tek), following the manufacturer's instructions. Final elutions were done in a total volume of 150 µl elution buffer. The presence of DNA was checked on an agarose gel. The internal transcribed spacer (nrITS) was amplified with primers ITS1F (GARDES & BRUNS, 1993) and ITS4 (WHITE et al., 1990), while 28S nrLSU region was amplified with primers LR0R and LR5 (VILGALYS & HESTER, 1990). For PCR reactions puReTaq Ready-To-Go PCR Beads™ (GE Healthcare) were used following manufacturer's instructions, adding 1 µl of genomic DNA to a final reaction volume of 25 µl. PCR conditions were: 5 min initial denaturation at 94-95°C followed by 40 cycles of: 45-60 seg denaturation at 95°C, primer annealing at 54°C for 30 seg, and extension at 72°C for 30 seg, followed by a final extension step of 10 min at 72°C. Sequences were obtained in the laboratories of StabVida (Lisboa) or Macrogen (Amsterdam) with the same primers used in the amplification reaction.

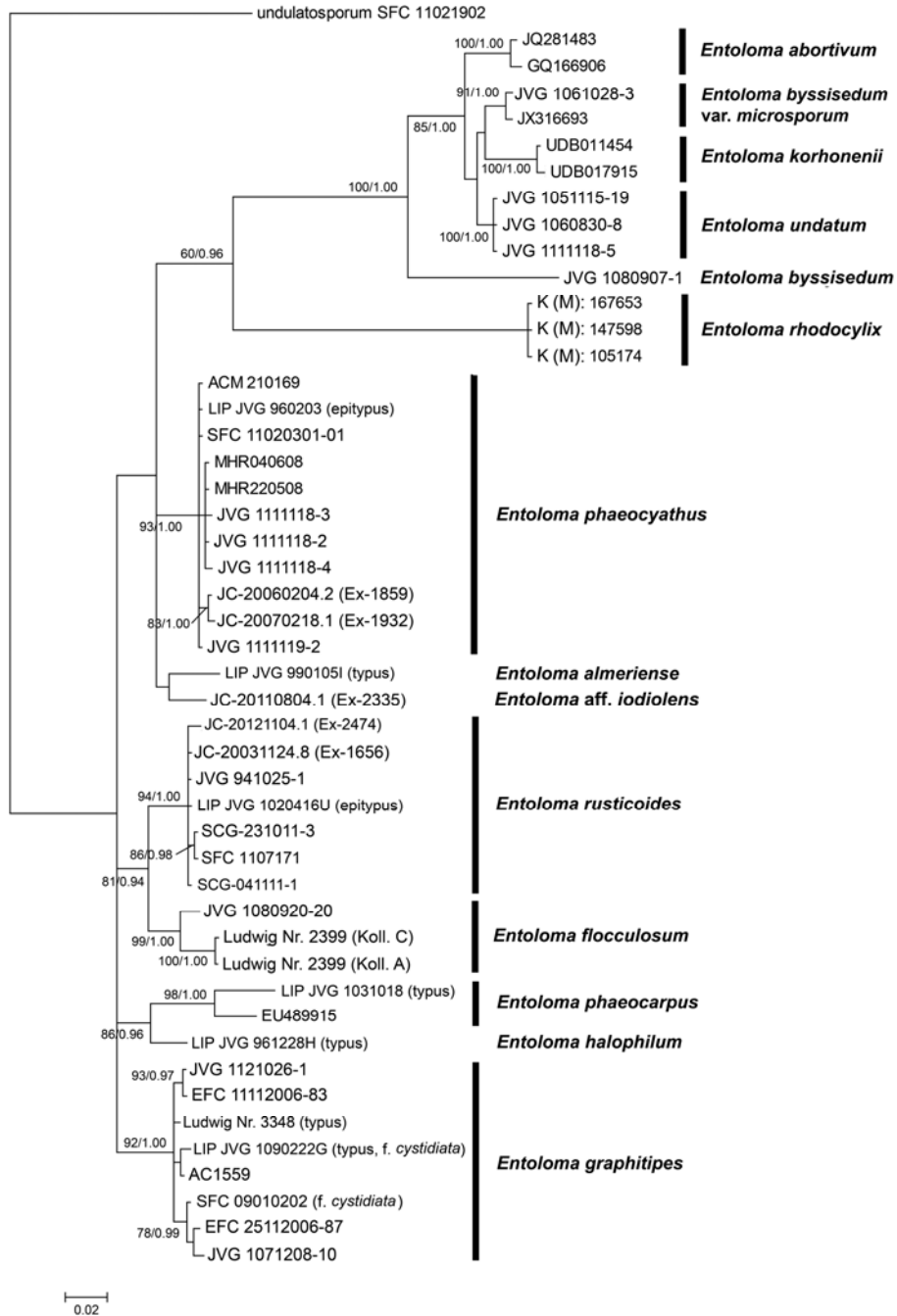
### C) PHYLOGENETIC ANALYSES

Chromatograms were visually checked searching for putative reading errors, and the sequences end-trimmed at both ends, then aligned with the Clustal W algorithm, as implemented in MEGA 5 software (TAMURA et al., 2011) and manually adjusted. The final alignment included 129/270 (ITS1), 1/117 (5.8S), 130/280 (ITS2), and 64/856 (28S) variable sites. The aligned loci were subjected to MrModeltest 2.3 (NYLANDER, 2004) in PAUP\* 4.0b10 (SWOFFORD, 2001). Model HKY+ $\Gamma$  was selected for ITS1 and ITS2, and JC for 5.8S partition, while GTR+I+ $\Gamma$  was selected for the 28S nLSU analysis. These models were implemented in MrBayes 3.1 (RONQUIST & HUELSENBECK, 2003), where a Bayesian analysis was performed (ITS1-5.8S-ITS2 data partitioned, two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after about 560,000 (ITS) and 630,000 (28S nLSU) generations: the standard deviation having fallen below 0.01. Finally a full search for the best-scoring maximum likelihood tree was performed in RAxML (STAMATAKIS, 2006) using the standard search algorithm (ITS1-5.8S-ITS2 data partitioned, 2000 bootstrap replications). Significance threshold was set above 0.9 for posterior probability (PP), and 70% bootstrap proportions (BP).

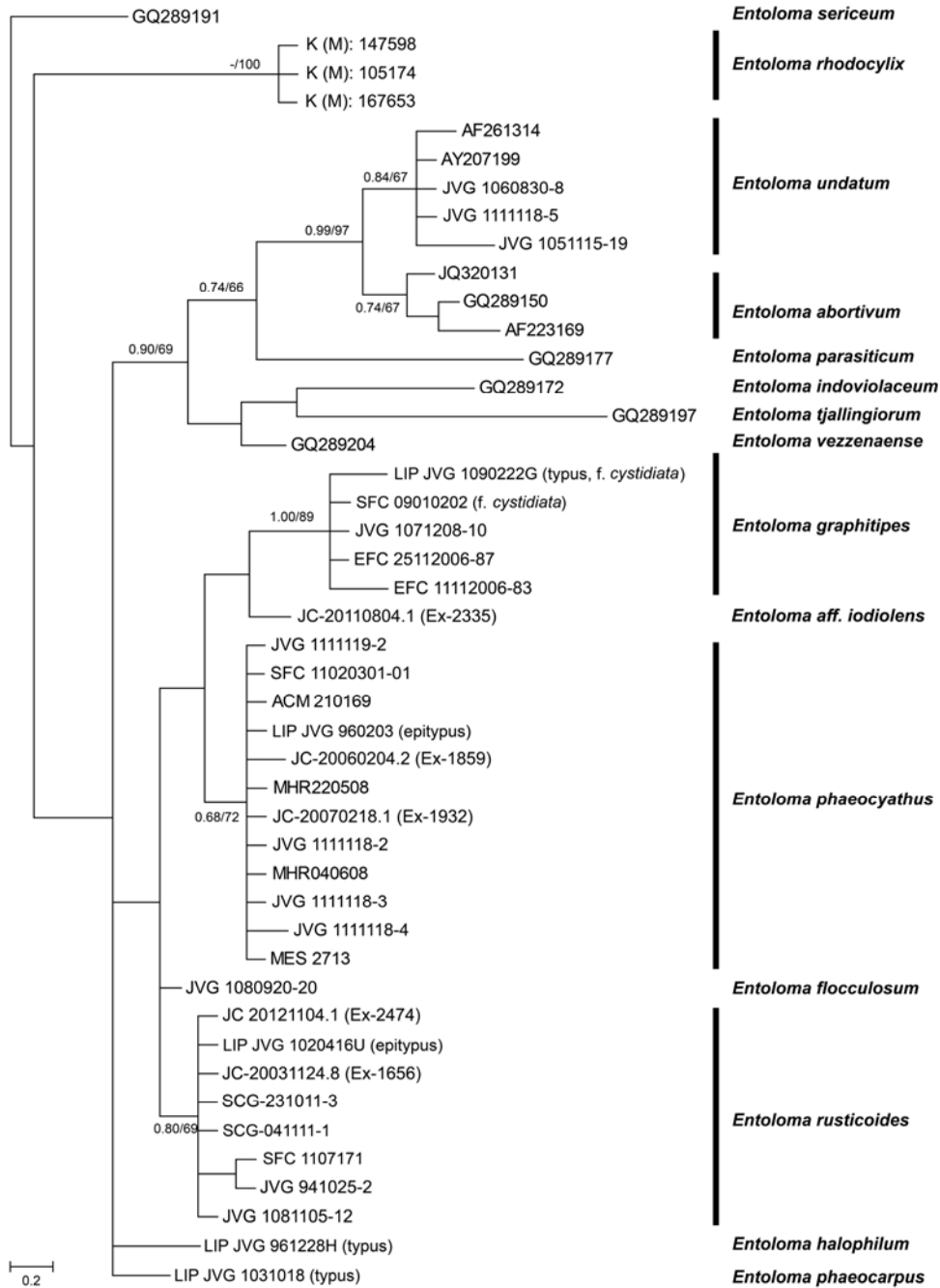
### PHYLOGENETIC RESULTS

Phylogenetic inference based on ITS (Fig. 1) and 28S nrLSU (Fig. 2) was consistent at the species level. Groups presenting a monophyletic origin were studied and described as independent taxa, and the diagnostic features of each group were evaluated accordingly. Sequences of *Entoloma rusticoides* and associated taxa seemed to be closely related to the *Claudopus* lineage, but probably independent from it. Whether or not they constitute a new section should be investigated with a different, multigenic approach. The cystidiate form of *E. graphitipes* does not seem to differ significantly from the typical form when ITS or 28S nrLSU markers are compared, and hence, both are here considered conspecific.

The new species *Entoloma halophilum*, *E. phaeocarpum* and *E. almeriense* are proposed to accommodate the lineages matching none of the previously known species in these groups. *E. korhonenii* seem to be related to the *Claudopus* lineage, while *E. rhodocylix* seems to have an intermediate position between this lineage, subgenus *Nolanea* and *E. rusticoides* and related taxa.



**Fig. 1.** ITS Bayesian consensus phylogram obtained in MrBayes 3.1. The values next to nodes represent Bayesian posterior probabilities and maximum likelihood bootstrap proportions. Only nodes significantly supported by both methods are shown.



**Fig. 2.** 28S nLSU Bayesian consensus phylogram obtained in MrBayes 3.1. The values next to nodes represent Bayesian posterior probabilities and maximum likelihood bootstrap proportions. Only nodes significantly supported by both methods are shown.



*Entoloma rusticoides* (epitypus). Photo J. Vila.



*Entoloma rusticoides* (JC-20121104.1 (Ex-2474)). Photo J. Carbó.

KEY TO THE SPECIES

- 1. Cheilocystidia present .....2
- 1. Cheilocystidia absent.....6
- 2. Clamps present .....9. *E. rhodocylix*
- 2. Clamps absent.....3
- 3. Spores 8.1–10 × 6.9–8.9 μm, X<sub>m</sub>= 9 × 7.8 μm; stipe filiform, up to 1 mm in diameter; under *Rubus*.....3a. *E. graphitipes* f. *cystidiatum*
- 3. Spores bigger; stipe broader, 2–3 mm in diameter .....4
- 4. Pileus with brown or red-brown tinges; spores 9.3–11.2 x 8.7–10.6 μm, X<sub>m</sub>= 10.2 × 9.6 μm; basidia mono-, bi- or tetrasporic; on sandy saline soil..... 5. *E. halophilum*
- 4. Pileus lacking red tinges; spores slightly smaller; basidia tetrasporic, rarely bisporic; on sandy saline soil or not .....5
- 5. Pileus convex, not or weakly hygrophanous, tomentose-pruinose, grey; cheilocystidia 5–10 μm broad; on saline soil ..... 6. *E. almeriense*
- 5. Pileus convex to umbilicate, strongly hygrophanous, not tomentose but subsquamulose, brownish-grey to blackish-grey; cheilocystidia 8–15.5 μm broad; on different habitats..... 2. *E. phaeocyathus*
- 6. Clamps present .....7
- 6. Clamps absent.....9
- 7. Pileus pale greyish to whitish; spores heterodiametrical ..... 10. *E. muscoalpinum*
- 7. Pileus brownish; spores isodiametrical to subisodiametrical .....8
- 8. Pileus convex to umbilicate; spores isodiametrical, 7–9.5 x 6.5–9 μm ..... *E. rickenellaeformis* (not traited)
- 8. Pileus convex to flattened; spores subisodiametrical, 6.5–7.5 x 5–6.5 μm ..... *E. chelone* (not traited)
- 9. Spores subisodiametrical, Q= 1.07–1.31; pileus with tomentose-floccose surface ..... 4. *E. flocculosum*
- 9. Spores isodiametrical, Q= 1–1.2; pileus surface smooth to fibrillose or pruinose, rarely subsquamulose ..... 10
- 10. Pileus strongly hygrophanous, convex to umbilicate, very dark in well-hydrated specimens ..... 2. *E. phaeocyathus*
- 10. Pileus not or weakly hygrophanous, convex to flattened, paler ..... 11
- 11. Pileipellis with intraparietal and incrusting epiparietal pigment; lamellae with grayish-lilacinous tinges and intervenose; stipe central to eccentric ..... 7. *E. phaeocarpum*
- 11. Pileipellis with dominant incrusting epiparietal pigment; lamellae without lilacinous tinges; stipe central ..... 12
- 12. Pileus up to 20 mm in diam., with dark brown-grayish colour in well-hydrated specimens; stipe surface pilose to almost smooth ..... 1. *E. rusticoides*
- 12. Pileus up to 10 mm in diam., paler; stipe surface glabrous or slightly pruinose ..... 3. *E. graphitipes* f. *graphitipes*



## TAXONOMY

1. *Entoloma rusticoides* (Gillet) Noordel., Persoonia 11: 150 (1981)

EPITYPUS (DESIGNATED HERE). SPAIN: near Castell de Sant Jordi, l'Ametlla de Mar (Tarragona), alt. 15 m; among mosses and lichens, under *Cistus chusii* Dunal and *Rosmarinus officinalis* L., in basic soil, 16 Apr. 2002, leg. J. Vila and X. Llimona, LIP JVG 1020416U, isoepitypus in JVG 1020416-2.

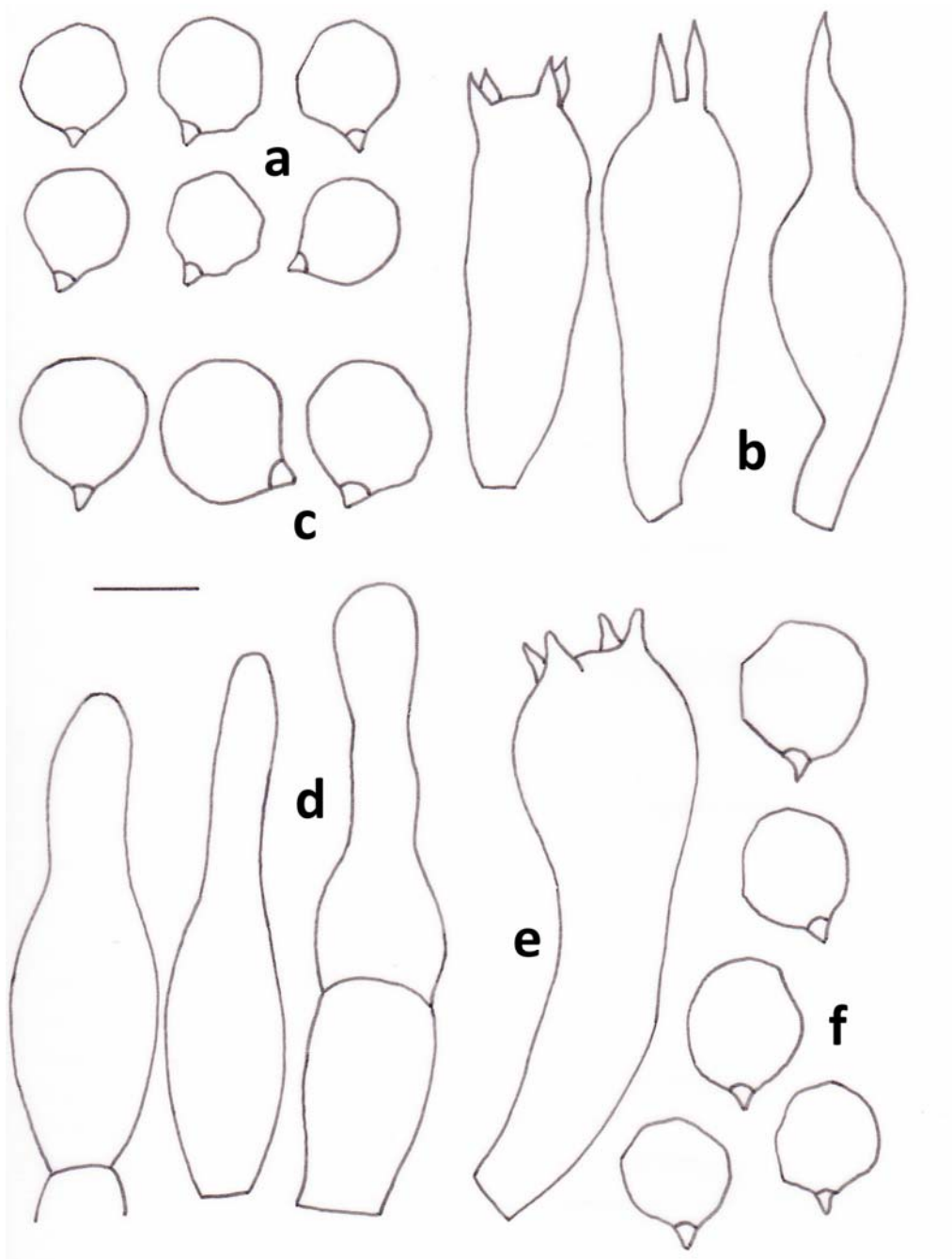
Pileus up to 20 mm in diam., convex or flattened-convex when young, somewhat umbilicated in the center, becoming more flattened but also umbilicated with age, not or only slightly hygrophanous, surface smooth or minutely pruinose-roughened, somewhat subsquamulose or fibrillose when dry, margin slightly translucently striate up to 2/3 of the radius in well-hydrated specimens; brownish-grey when moist, with the center darker and even blackish, with a light brownish colour with inconspicuous grayish tones when dry; margin straight or slightly curved, sometimes a bit wavy. Lamellae decurrent, rarely subdecurrent, arcuate to sub-straight, very distant, somewhat thick; grey or brownish-grey, lighter than the pileus, becoming pinkish-brown with age; edge of the same colour, a bit irregular; lamellulae abundant. Stipe central, up to 15 × 3 mm, cylindrical, straight or curved, similar in colour to the pileus, surface almost smooth or with a minute but abundant whitish pilosity if moist, apex pruinose, base not differentiated. Flesh very thin, brownish-grey; smell not remarkable or radish-like, taste not tested. Spores isodiametrical, less frequently subisodiametrical, 8–10.7 × 6.9–10.4 μm,  $X_m = 9.3 \times 8.7 \mu m$ ,  $Q = 1-1.2$ ,  $Q_m = 1.07$ , rounded or with 5-6 inconspicuous angles. Basidia tetrasporic, sometimes mono- or bisporic, subcylindrical to narrowly claviform, up to 45 × 12.8 μm (with sterigmata), clampless. Cystidia absent, rarely some sterile basidioloid elements are observed. Pileipellis with a cutis structure composed of cylindrical hyphae, 4–12 μm wide; brownish incrustant epiparietal pigment abundant, diffuse vacuolar pigment also present; subpellis with broader hyphae, up to 20 μm in diam. Clamp-connections not observed.

ADDITIONAL COLLECTIONS EXAMINED. SPAIN: near Castell de Sant Jordi, l'Ametlla de Mar (Tarragona), alt. 15 m; among mosses and lichens (*Fulgensia* sp.), in basic soil, 5 Nov. 2008, leg. J. Vila and X. Llimona, JVG 1081105-12 and JVG 1081105-19. *Ibid.*, among mosses and lichens, in basic soil, 23 Nov. 2011, leg. J. Vila and X. Llimona, JVG 1111123-3, JVG 1111123-4 and JVG 1111123-5. Timoneda d'Alfés, Alfés (Lleida), alt. 110 m; among grasses and lichens, near *Thymus* sp., in basic soil, 25 Oct. 1994, leg. J. Vila and X. Llimona, JVG 941025-1. Sant Fost de Campsentelles (Barcelona), alt. 100 m; among grasses, mosses and lichens, 17 Jul. 2011, leg. F. Caballero, SFC 1107171. Son Rossinyol, Coll d'en Rebassa, Palma (Mallorca), alt. 5 m; among grasses, in basic soil, 24 Nov. 2003, leg. J. Carbó, JC-20031124.8 (Ex-1656). Quatretonda (València), alt. 380 m; near *Cistus monspeliensis* L., in sandy soil, 23 Oct. 2011, leg. I. Garrido, SCG-231011-3. *Ibid.*, leg. J. Ormad, SCG-041111-1. Near Castellldans (Lleida), alt. 360 m; among mosses, in basic soil, 25 Oct. 1994, leg. J. Vila and X. Llimona, JVG 941025-2. La Fonollera, Torroella de Montgrí (Girona), alt. 10 m; under *Olea europaea* L. subsp. *sylvestris* (Mill.) Rouy ex Hegi and *Pistacia lentiscus* L., among *Thymus vulgaris* L. and *Helianthemum* sp., 4 Nov. 2012, leg. J. Carbó, JC-20121104.1 (Ex-2474).

2. *Entoloma phaeocyathus* Noordel., Persoonia 12(4): 461 (1985)

= *Eccilia tristis* Bres. in Saccardo (1891) non *Entoloma triste* (Velen.) Noordel.

EPITYPUS (DESIGNATED HERE). SPAIN: el Mustinyà, Puig de la Reina, Torroella de Montgrí (Girona), alt. 115 m; under *Quercus coccifera* L. and *Rosmarinus officinalis*, among mosses, in stony clay soil with basic reaction, 3 Feb. 1996, leg. J. Carbó, LIP JVG 960203, isoepitypus in JC-19960203.5 (Ex-511).



**Fig. 3.** *Entoloma rusticoides* (epitypus): a) spores; b) basidia. *E. rusticoides* (JVG 941025-1): c) spores. *E. phaeocyathus* (epitypus): d) cheilocystidia; e) basidia; f) spores. Scale bar = 8  $\mu$ m.

Pileus up to 15 mm in diam., variable in shape, some omphalioid specimens appear flat to depressed or infundibuliform, others are convex to hemispherical, hygrophanous, not translucently striate, surface with scarce to abundant fibrils, sometimes looking subsquamulose in dehydrated basidiomata; brownish-grey to blackish-grey, light brown or dark buff when dry; straight margin, slightly incurved or wavy in some specimens. Lamellae adnate to decurrent, distant, thick, grayish to brownish-grey with pinkish shades when mature; edge of the same colour or slightly darker, entire or irregular; lamellulae abundant. Stipe central, up to 25 × 4 mm, cylindrical, straight or somewhat curved, glabrous to pruinose depending on the specimen and environmental moisture; colour similar to the pileus. Flesh thin, grayish, brownish or whitish; smell not remarkable or slightly herbaceous, taste not defined. Spores isodiametrical, or subisodiametrical, 9.2–12 × 8.5–10.8 µm,  $X_m = 10.1 \times 9.2$  µm,  $Q = 1.03$ –1.2,  $Q_m = 1.1$ , with a roundish perimeter and 6–8 weak angles. Basidia tetrasporic, subclaviform to cylindrical, sometimes subglobose, up to 48.7 × 15.5 µm (without sterigmata), clampless. Lamellae edge heterogeneous. Cheilocystidia polymorphic, clavate, some of them articulated with widened apex, others sublageniform or almost cylindrical, up to 65 × 15.5 (base) × 8 (apex) µm; interestingly, some of the collections studied did not present cheilocystidia. Pileipellis with cylindrical or subclaviform hyphae, up to 100 µm long, with widened apical elements, up to 19 µm in diam.; trichodermis structures observed, especially in the center of the pileus. Subpellis and pileitrama with elongated hyphae, up to 200 × 16 µm, constricted at septa, mixed with thinner hyphae, up to 7 µm in diam; incrustant epiparietal pigment brown, often zebra-striped, present in all structures studied, although scarce in lamellae trama; intraparietal pigment abundant, brown, vacuolar pigment present, scarce. Clamp-connections absent.

ADDITIONAL COLLECTIONS EXAMINED. SPAIN: Mas Ventós, Pantaleu, Palau-sator (Girona), alt. 40 m; among grasses, in clay soil, 18 Feb. 2007, leg. J. Carbó, JC-20070218.1 (Ex-1932).- La Fonollera, Torroella de Montgrí (Girona), alt. 10 m; under *Olea europaea* subsp. *sylvestris*, among grasses and *Thymus vulgaris*, in sandy soil, 4 Feb. 2006, leg. J. Carbó, JC-20060204.2 (Ex-1859).- Sant Fost de Campsentelles (Barcelona), alt. 100 m; among grasses, mosses and lichens, in acid soil, 1 Mar. 2011, leg. F. Caballero, SFC 11020301-01.- *Ibid.*, 19 Nov. 2011, leg. S. Català, J. Vila and F. Caballero, SFC 111119-1 and JVG 111119-2.- El Puerto de la Quesera, Riaza (Segovia), alt. 1770 m; among grasses and mosses, 4 Jun. 2008, leg. M. Higuelmo, MHR040608.- Sa Planassa, Cadaqués (Girona), alt. 105 m; in a litoral xerophytic meadow, among grasses, in acid soil, 18 Nov. 2011, leg. J. Vila, X. Llimona, S. Català and I. Garrido, JVG 111118-2, JVG 111118-3 and JVG 111118-4.- Font Roja d'Alcoi (Alacant), alt. 910 m; under *Pinus halepensis* Mill., in basic soil, 1 Jan. 1996, leg. A. Conca, det. R. Mahiques, MES 2713.- Saldaña (Palencia); among grasses, under *Quercus ilex* L., in acid soil, 22 May 2008, leg. M. Higuelmo, MHR220508.- *Ibid.*, 24 May 2008, leg. M. Higuelmo, MHR240508.- El Saler (València), alt. 0–5 m; among grasses, near *Pinus halepensis* and *P. canariensis* C. Sm., in sandy soil, 28 Dec. 2010, leg. A. Conca and M. Micó, ACM 210169.

Differences between *Entoloma rusticoides* and *E. phaeocyathus* are not always evident, and the short original diagnoses of both species do not help to discriminate between them:

a) *Entoloma rusticoides*: "*Chapeau membraneux, convexe-ombiliqué, strié, glabre, hygrophane, brun, isabelle à l'état sec, livide. Feuilletts d'abord adhérents, puis long. décurrents, assez peu nombreux, gris. Pied fistuleux, égal, glabre, lisse, concolore au chapeau. Espèce petite, ténace. Habitat: dans les parties herbeuses des bois*" (GILLET, 1876).

Translation of the original description: Membranaceous pileus, convex-umbilicated, striated, glabrous, hygrophanous, brown, isabelle (when dry), livid. Lamellae first adherent, then longly decurrent, few in number, grey. Stipe fistulose, equal, glabrous,

smooth, same colour as the pileus. Minute and tenacious species. Habitat: in grassy parts of the woods.

b) *Entoloma phaeocyathus*: "Pileo submembranaceo, convexo subcampanulato, umbilicato, margine undulato-crispato, striato, udo, glabro, brunneo, 1-1,5 cm lato; lamellis distantibus, crassis, carneo-brunneis, latis, arcuato-decurrentibus; stipite e farcto cavo, concolore, glabro, basi attenuato, 2-3 cm longo, 2 mm circiter crasso; carne subconcolori, odore grato spirituosissimo-dulci, sapore nullo; sporis 6-7 angulatis, subglobosis, 8-10  $\mu$ m diam. Habi. in horto botanico ad vasa in Tepidariis" (BRESADOLA in SACCARDO, 1891, sub *Eccilia tristis*).

Translation of the original description: Pileus submembranaceous, convex to subcampanulate, umbilicate, margin undulate-crispate, striate, moist, glabrous, brownish, 1–1.5 cm wide; lamellae distant, thick, buff to brown in colour, broad, arcuate-decurrent; stipe hollow, same colour as the pileus, glabrous, compressed at the base, 2–3 cm long, about 2 mm thick; flesh nearly the same colour, pleasant spirituous/sweet smell; taste indistinct; spores with 6-7 angles, subglobose, 8–10  $\mu$ m in diam. Habitat: pots in greenhouse of a botanic garden.

Despite their similarity, the many collections analyzed here allow us to establish some differences between the most typical samples of each species, for example, a somewhat larger size and a less umbilicated shape in *Entoloma rusticoides*. The colour of *E. rusticoides* is also typically a little more brownish, but some specimens with an almost identical colour to that observed in *E. phaeocyathus* can be found. The abundance of pruina on the stipe surface seems not to be a robust character in either taxa. At the microscopic level, the presence of cheilocystidia in *E. phaeocyathus* has been often taken as a diagnostic feature, but here we observed some collections [e.g. JC-20060204.2 (Ex-1859), MHR040608 or MHR220508] where these are not developed, causing the lamellar edge to appear fully fertile. The size and shape of spores is very similar in both species. Finally, *E. rusticoides* was exclusively found on basic soil, commonly in dry and sunny places at low altitude. *E. phaeocyathus* was instead collected from both acidic and basic soils, and can be found also at higher altitude, in open ruderal soils, forest clearings. Both species prefer Mediterranean habitats.

*Entoloma graphitipes* can be differentiated by its smaller size, the lighter brownish colour, and its smaller spores. *E. halophilum* presents more brownish lamellae when young, basidia are mono-, bi- or tetrasporic, and can be found in salty soils. *E. flocculosum* exhibits a densely pruinose-floccose pileus surface when young, immature lamellae whitish, and spores more subspherical. See also comments regarding *E. almeriense*.

### 3. *Entoloma graphitipes* E. Ludw., Pilzkompodium. Band 2

(Beschreibungen): 290 (2007) f. *graphitipes*

= *E. flocculosum* ss. Vila & Caballero (2007)

Pileus up to 10 mm in diam., flattened to depressed, a little more depressed with age, slightly hygrophanous, in well-hydrated specimens translucently striate up to half of the radius, smooth or somewhat rough in the center, sometimes with a minute whitish pruina; grayish-brown or brown, with the central disc distinctly darker; margin lighter, straight or slightly incurved when young. Lamellae subdecurrent to strongly decurrent,



*Entoloma rusticoides* (JVG 1111123-5). Photo J. Vila.



*Entoloma phaeocyathus* (epitypus). Photo J. Carbó.



*Entoloma phaeocyathus* (JVG 1111119-2). Photo J. Vila.



*Entoloma phaeocyathus* (JVG 1111118-4). Photo J. Vila.

distant, thick, somewhat ventricose in some specimens; brownish or light grayish-brown, more pinkish after spore maturation; edge of the same colour, entire; lamellulae present. Stipe central, up to  $15 \times 2$  mm, cylindrical or slightly compressed at the base, straight to curved; colour similar to the pileus or somewhat darker; surface smooth, glabrous or with an inconspicuous whitish pruina, more evident at the apex. Flesh very thin, light grayish-brown, darker in the uppermost part of the stipe; smell fungic. Spores isodiametrical to subisodiametrical,  $8.3\text{--}9.3 \times 7.2\text{--}8.3$   $\mu\text{m}$ ,  $X_m = 8.8 \times 7.7$   $\mu\text{m}$ ,  $Q = 1.1\text{--}1.18$ ,  $Q_m = 1.15$ , with weak angles and the perimeter sometimes looking roundish. Basidia tetrasporic, claviform to narrowly claviform, up to  $43 \times 13.3$   $\mu\text{m}$  (with sterigmata), clampless. Cystidia absent. Pileipellis with a cutis structure composed of thin hyphae,  $2\text{--}8$   $\mu\text{m}$  wide; abundant incrustant epiparietal pigment, brownish. Subpellis differentiated, with thicker hyphae,  $9.6\text{--}28$   $\mu\text{m}$  wide, rarely up to  $50$   $\mu\text{m}$  in diam., hyaline or with diffuse parietal brownish pigment. Clamp-connections absent.

COLLECTIONS EXAMINED. GERMANY: Kinderspielplatz an der John-Locke-Str. 29, Berlin-Lichtenrade; on bare sandy soil, partly between grasses, some fruit bodies growing on an adjacent old fireplace, 22 May 1982, leg. E. Ludwig, Ludwig Herb. Nr. 3348 (M) (TYPUS). SPAIN: Ortigosa de Cameros (la Rioja), alt. 1100 m; among mosses, near *Quercus ilex*, in basic soil, 26 Oct. 1991, leg. A. Caballero, AC1559.- Serrat de l'Artigot, la Quar (Barcelona), alt. 780 m; among grasses, lichens and mosses, near *Satureja montana* L., in basic soil, 11 Nov. 2006, leg. J. Vila and F. Caballero, JVG 1061111-1 and EFC 11112006-83.- Pla de Busa, Navès (Lleida), alt. 1250 m; among grasses, mosses and lichens, in basic soil, 25 Nov. 2006, leg. J. Vila and F. Caballero, JVG 1061125-1 and EFC 25112006-87.- San Cristóbal, Busturia (Bizkaia), alt. 0-5; in a dune, on sandy soil, with small plants not identified, 8 Dec. 2007, leg. J. Fernández and F. Hidalgo, JVG 1071208-10.- *Ibid.*, 29 Nov. 2007, leg. J. Fernández, JVG 1071129-10.- Can Soteres, el Bruc (Barcelona), alt. 530 m; among mosses and lichens, in basic soil, 26 Oct. 2012, leg. J. Vila and X. Llimona, JVG 1121026-1.

Because of its shape, *Entoloma graphitipes* f. *graphitipes* could be confused with *E. rhodocylix*, but the latter can be easily differentiated by its clamped hyphae (NOORDELOOS, 1992; LUDWIG, 2007), not observed in any of the studied species related to the lineage *rusticoides-phaeocyathus*. See also comments regarding *E. halophilum* and *E. phaeocyathus*.

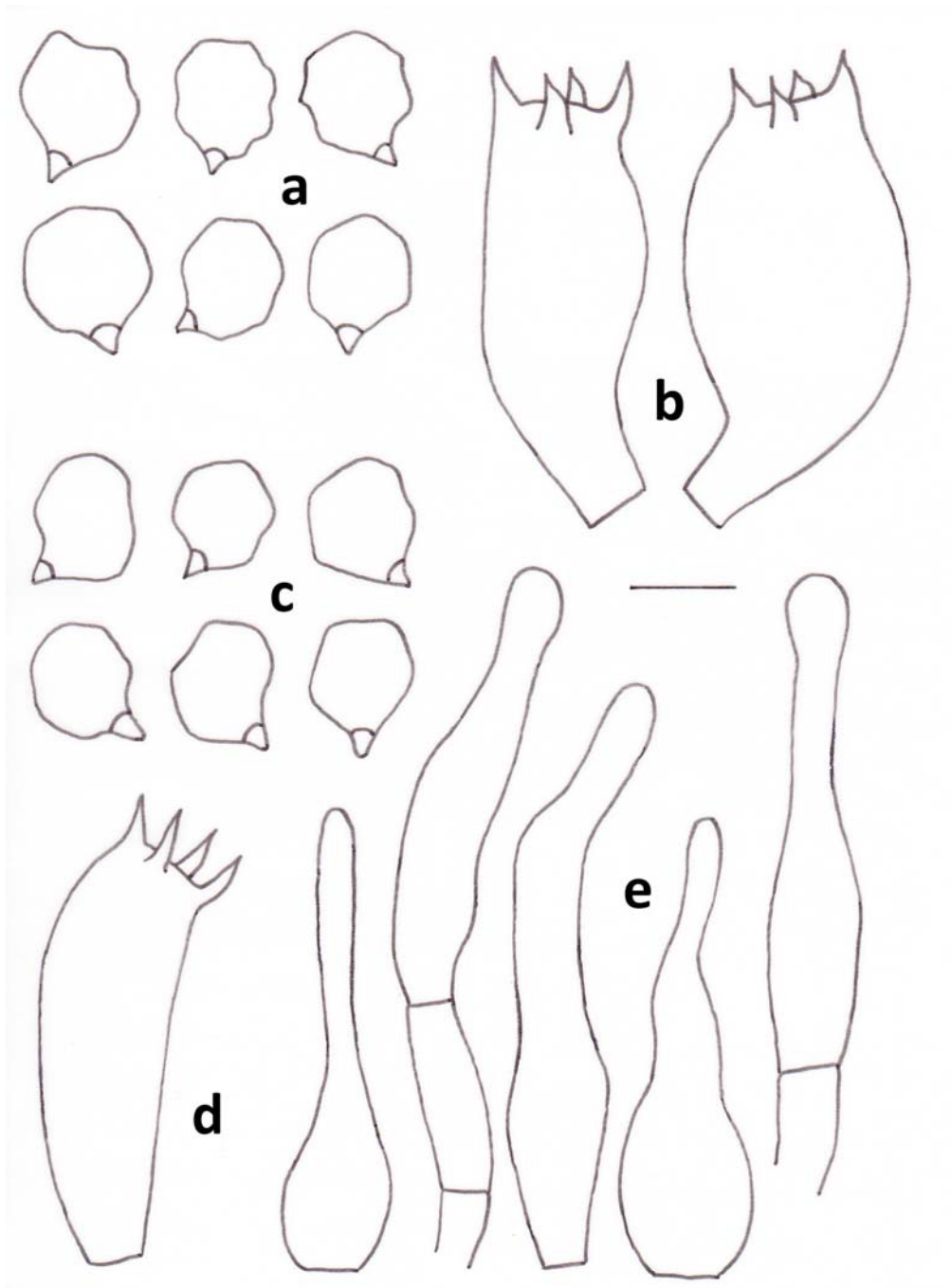
### 3a. *Entoloma graphitipes* f. *cystidiatum* F. Caball., Vila & Català f. nov.

Mycobank MB 807473

ETYMOLOGY. *cystidiatum*, because of the presence of cystidia on the edge of the gills.

TYPUS. SPAIN: Can Romegosa, Sant Fost de Campsentelles (Barcelona), alt. 140 m; under *Rubus ulmifolius* Schott, near *Quercus ilex* and *Pinus pinea* L., in acid soil, 22 Feb. 2009, leg. F. Caballero, LIP JVG 1090222G, *isotypus* SFC 090222-1.

ORIGINAL DIAGNOSIS: Pileus up to 10 mm in diam., first convex, then umbilicated, somewhat hygrophanous, translucently striate in well-hydrated specimens, fibrillose-silky when dry; light grayish-brown, especially in the central disc of the pileus, lighter near the margin, which is straight. Lamellae adnate, with a small decurrent tooth, strongly decurrent in mature specimens, distant, thick; creamy-grayish or light grayish-beige, pale grayish-brown with pinkish shades when mature; edge of the same colour, slightly eroded; lamellulae present. Stipe central, filiform, up to  $25 \times 1$  mm, cylindrical, widened at apex and at the base, straight or curved; beige, light brown or grayish-brown, paler than the pileus from the apex to approximately  $2/3$  of the length, base darker; surface smooth, somewhat pruinose at the apex, base



**Fig. 4.** *Entoloma graphitipes* f. *graphitipes* (JVG 1071129-10): a) spores; b) basidia. *E. graphitipes* f. *cystidiatum* (*typus*): c) spores; d) basidia; e) cheilocystidia. Scale bar = 8  $\mu$ m.





*Entoloma graphitipes* f. *graphitipes* (JVG 1061125-1). Photo J. Vila.



*Entoloma graphitipes* f. *graphitipes* (JVG 1121023-1). Photo J. Vila.



*Entoloma graphitipes* f. *graphitipes* (AC 1559). Photo A. Caballero.



*Entoloma graphitipes* f. *cystidiatum* (typus). Photo F. Caballero.

tomentose. Flesh thin, scarce, grayish-brown in the pileus, lighter in the stipe; smell slightly farinaceous when rubbed, taste not remarkable. Spores isodiametrical to subisodiametrical,  $8.1\text{--}10 \times 6.9\text{--}8.9 \mu\text{m}$ ,  $X_m = 9 \times 7.8 \mu\text{m}$ ,  $Q = 1\text{--}1.3$ ,  $Q_m = 1.16$ , with 5-7 inconspicuous angles. Basidia tetrasporic, rarely bisporic, subcylindrical to claviform, up to  $34 \times 15.9 \mu\text{m}$  (without sterigmata), clampless. Cheilocystidia (lamella edge heterogeneous) polymorphic, cylindrical, sublageniform to lageniform,  $45.7\text{--}57.3 \times 4.1\text{--}13.9 \mu\text{m}$ , with 0-2 septa. Pileipellis with a cutis structure composed of thick parallel hyphae, up to  $20 \mu\text{m}$  in diam. Subpellis with thick hyphae, constricted at septa, mixed with more narrow ones,  $2\text{--}4 \mu\text{m}$  wide, epiparietal pigment minutely incrusting. Clamp-connections not observed.

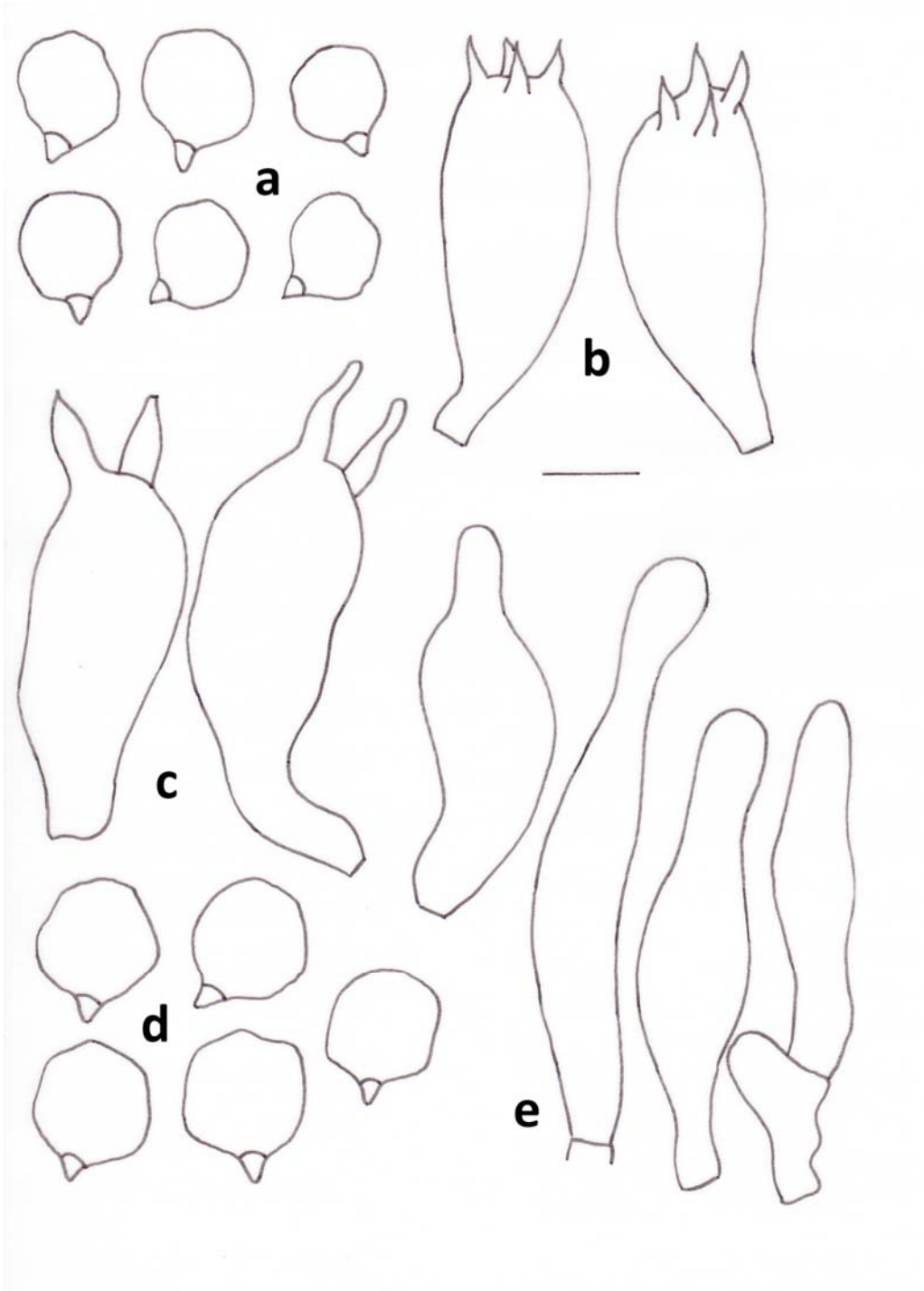
ADDITIONAL COLLECTIONS EXAMINED. SPAIN: Can Romegosa, Sant Fost de Campsentelles (Barcelona), alt. 140 m; under *Rubus ulmifolius*, near *Quercus ilex* and *Pinus pinea*, in acid soil, 19 Apr. 2008, leg. F. Caballero, SFC 080419-1.- Mas Llombart, Sant Fost de Campsentelles (Barcelona), alt. 140 m; near *Rubus ulmifolius* and *Arbutus unedo* L., among grasses, in acid soil, 2 Jan. 2009, leg. F. Caballero, SFC 09010202.

*Entoloma graphitipes* f. *cystidiatum* differs from the type because of: (i) basidiomata with lighter colour, especially in the stipe, (ii) presence of abundant cheilocystidia, (iii) spores slightly bigger, and (iv) absence of trichodermal elements in the pileipellis. See also comments regarding *E. graphitipes* f. *graphitipes* and *E. phaeocyathus*.

#### 4. *Entoloma flocculosum* (Bres.) Pacioni, Micol. Veg. Medit. 2(2): 148 (1988)

Pileus up to 10 mm in diam., convex-flattened to flat, depressed in the central disc, especially with age, neither hygrophanous nor translucently striate; surface with an abundant whitish pruina when young, that turns brownish and floccose to subsquamulose in mature specimens, more dense in the central disc; under the pruina, homogeneously more or less dark brownish, sometimes with grayish shades; margin straight to slightly curved. Lamellae adnate to subdecurrent, distant, thick, first whitish to pale brownish, then pinkish-brown; edge of the same colour, entire or a bit irregular; lamellulae not abundant. Stipe central, up to  $15 \times 3 \text{ mm}$ , cylindrical, straight or slightly curved; clearly paler than the pileus, pale brownish to almost whitish; surface crowded by fine whitish fibrils, progressively disappearing with age or when rubbed; apex not differentiated, base whitish and tomentose. Flesh thin, cream or brownish; smell indefinite; taste not tested. Spores subisodiametrical, rarely isodiametrical,  $8.3\text{--}10.1 \times 7.5\text{--}9.1 \mu\text{m}$ ,  $X_m = 9.2 \times 8.1 \mu\text{m}$ ,  $Q = 1.07\text{--}1.31$ ,  $Q_m = 1.21$ , commonly with a rounded perimeter and poorly differentiated angles. Basidia tetrasporic, claviform to subclaviform, up to  $35 \times 12.3 \mu\text{m}$  (with sterigmata), clampless. Cystidia or sterile cells absent. Pileipellis with a cutis structure composed of cylindrical hyphae,  $3.2\text{--}8 \mu\text{m}$  wide, becomes a trichodermis, especially at the center of the pileus; incrustant epiparietal pigment very abundant, brownish, often zebra-striped or forming thick plates; subpellis composed by thicker hyphae, up to  $28 \mu\text{m}$  in diam., claviform to subfusiform in shape. Clamp-connections not observed.

COLLECTIONS EXAMINED: GERMANY. Naturschutzgebiet bei Lebus, Brandenburg; am Wegrand zwischen *Deschampsia* P. Beauv. sowie auf dem Gelände der Naturschutzstation auf nacktem, mit Ziegelsplitt versetztem Kies, 2 Sep. 1994, leg. W. Dieckow, Ludwig Herb. Nr. 2399 (Koll. A) (M).- S-Bahngelände bei Bahnhof Priesterweg, Tempelhof, Berlin; zwischen *Polytrichum* Hedw. unter Pappel und Birke auf sonnenexponiertem Sandboden, 12 Oct. 1991, leg. A. Rubner, Ludwig Herb. Nr. 2399 (Koll. C) (M). SPAIN: Serrat de l'Artigot, la Quar (Barcelona), alt. 780 m; among lichens and mosses, under *Satureja montana*, near *Buxus sempervirens* L., in basic soil, 20 Sep. 2008, leg. J. Vila and F. Caballero, JVG 1080920-20.



**Fig. 5.** *Entoloma flocculosum* (JVG 1080920-20): a) spores; b) basidia. *E. halophilum* (*typus*): c) basidia; d) spores; e) cheilocystidia. Scale bar = 8  $\mu$ m.

5. *Entoloma halophilum* F. Caball., J. Carbó, Vila & Català sp. nov.

MycoBank MB 807469

ETYMOLOGY. From the greek words *halo* = salt and *philo* = loving, preferring.

TYPUS. SPAIN: Las Salinas de San Miguel, San Miguel de Cabo de Gata (Almería), alt. 0-5 m; under *Thymelaea hirsuta* (L.) Endl., *Thymus* sp., *Arthrocnemum fruticosum* (L.) Moq. and *Lygeum spartum* (L.) Kunth., in fossil sand dunes parallel to the coast, in saline soil, 28 Dec. 1996, leg. J. Carbó, LIP JVG 961228H, *isotypus* in JC-19961228.3 (Ex-688).

ORIGINAL DIAGNOSIS: Pileus up to 20 mm in diam., convex to flat-convex, depressed in the center in fully mature specimens, hygrophanous, not translucently striate; surface felt-like, fibrillose-squamulose with age, more evident at the central disc; dark grayish-brown, specially from the margin to the half of the radius, showing pale brownish tones in the central disc, turning paler, grayish-white, when dry; margin straight. Lamellae decurrent or adnate with a small decurrent tooth, distant, somewhat thick, with very subtle veins on either side; brownish, darkening and with a salmon tone with maturation; lamellulae abundant. Stipe central, up to 20 × 3 mm, cylindrical, straight, fistulose; of the same colour as the lamellae; smooth surface, base not differentiated. Flesh thin, brownish; smell not defined, taste not tested. Spores isodiametrical, (8.5)–9.3–11.2–(12.5) × (8)–8.7–10.6–(12) μm,  $X_m = 10.2 \times 9.6$  μm,  $Q = 1-1.17$ ,  $Q_m = 1.06$ , with an almost rounded perimeter and 6-8 weak angles. Basidia mono-, bi- or tetrasporic, subclaviform to claviform, up to 40 × 15 μm (without sterigmata), with thickened walls (sclerobasidia), clampless. Cheilocystidia (lamella edge heterogeneous) polymorphic, sublageniform or lageniform, cylindrical, claviform, occasionally ovoid, up to 47 × 6 (apex)–15 (base) μm, not septated. Pileipellis with a cutis structure composed of cylindrical to subclaviform hyphae, up to 8 μm in diam., with terminal cells widened, up to 18 μm in diam.; the pileipellis becomes a trichodermis at the center of the pileus; incrustant epiparietal pigment abundant, brownish. Clamp-connections not observed.

*Entoloma halophilum* is characterized by its (i) small size of basidiomata, (ii) convex or even umbilicated pileus, (iii) colourful, very dark basidiomata, (iv) big, isodiametrical spores ( $X_m = 10.2 \times 9.6$  μm), (v) presence of an incrustant epiparietal pigment, and (vi) habitat in sandy saline soil. *E. rusticoides* differs by its overall more robust appearance, pileus translucently striate in well-hydrated specimens, smaller spores ( $X_m = 9.3 \times 8.7$  μm) and absence of cheilocystidia. *E. chelone* Noordel. & E. Horak differs by the different appearance of the basidiomata, these being more fleshy and flattened, as well as for its smaller spores (6.5–7.6 × 5–6.5 μm) (NOORDELOOS, 2004), cheilocystidia absent, and fruiting in the subalpine belt, under *Picea* and *Alnus*. *E. flocculosum* does not present cheilocystidia and has smaller spores ( $X_m = 9.2 \times 8.1$  μm). *E. korhonenii* Noordel. differs by its bigger basidiomata, absence of cheilocystidia and presence of clamp-connections (NOORDELOOS, 2004). *E. phaeocyathus* can present a depressed pileus similar to that of *E. halophilum*, but differs from it because of the spores iso- or subsodiametrical, and differently shaped cheilocystidia (claviform, articulated, rarely sublageniform), as well as for the more abundant intraparietal pigment. References to *E. phaeocyathus* in COURTECUISSÉ (1993) remind us very much of *E. halophilum*, especially because of the overall appearance of the basidiomata, the occasional presence of bisporic basidia, the shape



*Entoloma flocculosum* (JVG 1080920-20). Photo J. Vila.



*Entoloma halophilum* (typus). Photo J. Carbó.

of cheilocystidia, and the type of habitat of some of the collections described by the author: seaside dunes with halophilous vegetation.

6. *Entoloma almeriense* J. Carbó, F. Caball., Vila & Català sp. nov.

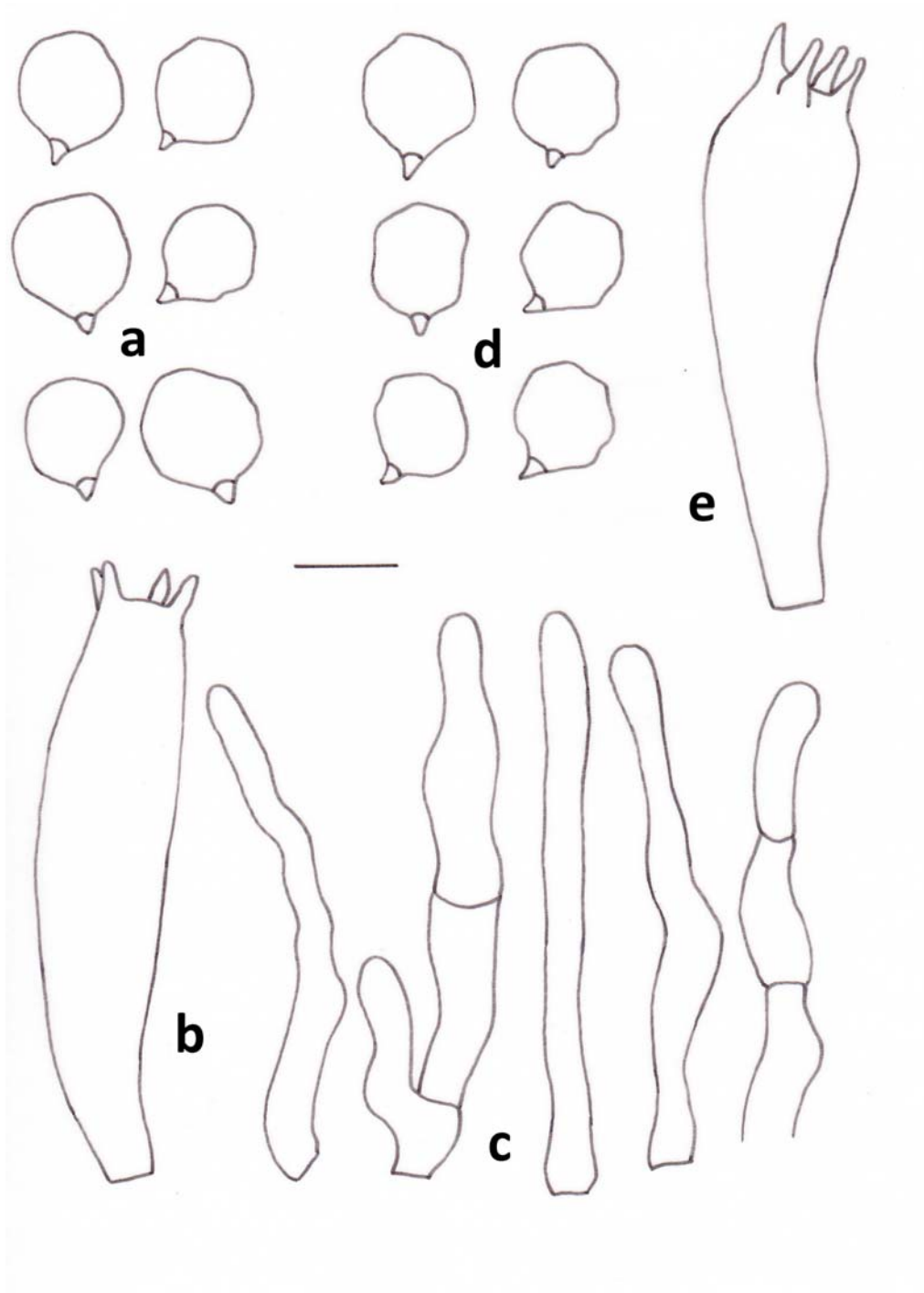
MycoBank MB 807470

ETYMOLOGY. From the geographical name Almería, the province of SW Andalucía (SW Spain).

TYPUS. SPAIN. Laguna del rio Antas, Puerto Rey-Pueblo Laguna, Vera (Almería), alt. 0-5 m; under *Thymelaea hirsuta*, *Arthrocnemum macrostachyum* (Mor.) C. Koch, *Atriplex halimus* L. and *Phragmites australis* (Cav.) Steud., in saline soil, 5 Jan. 1999, leg. J. Carbó, LIP JVG 990105I, *isotypus* in JC-19990105.4 (Ex-1037).

ORIGINAL DIAGNOSIS: Pileus up to 17 mm in diam., convex, somewhat hygrophanous, not translucently striate, with a pruinose-tomentose surface; grey, greyish-brown when well-hydrated; margin slightly curved. Lamellae adnate to decurrent, distant, moderately thick, ventricose, light grey, with pinkish tones when mature; edge of the same colour, entire; lamellulae not abundant. Stipe central, up to 25 × 2 mm, cylindrical, slightly curved, of the same colour as the pileus; surface with abundant whitish fibrils, longitudinally finely striated; apex slightly pruinose, base not differentiated. Flesh thin; smell and taste unknown. Spores isodiametrical, (8)–8.7–10.1–(11.5) × (7)–8–9.4–(11) μm,  $X_m = 9.4 \times 8.7$  μm,  $Q = 1-1.2$ ,  $Q_m = 1.08$ , with a rounded perimeter and 6-8 inconspicuous angles. Basidia tetrasporic, rarely bisporic, subcylindrical or narrowly claviform, up to 45 × 12 μm (without sterigmata), clampless. Cheilocystidia (lamella edge heterogeneous) cylindrical, sinuose, rarely sublageniform, (25)–30–50–(83) × 5–10 μm, septated. Pileipellis with a cutis structure composed of cylindrical or fusiform hyphae, 30–80 × 8–15 μm, with slightly broader terminal elements, up to 20 μm in diam., that evolves to a trichoderm; mixed pigment, intraparietal light brown, and minutely incrusting dark brown. Clamp-connections not observed.

*Entoloma almeriense* is characterized by its (i) greyish to brownish-grey pileus with tomentose surface, not translucently striate, (ii) distant lamellae, ventricose, grayish, (iii) stipe of the same colour of the pileus, slightly fibrillose, (iv) presence of narrow cheilocystidia, and (v) mixed pigment, intraparietal and minutely incrusting. *E. flocculosum* presents similar macro- and microscopic features, but differs by its more brownish colour, absence of cheilocystidia, and presence of incrustant epiparietal pigment only. *E. phaeocyathus* displays darker tones when well-hydrated, spores are slightly bigger, and cystidia present a different, thicker shape. *E. halophilum* is differentiated by its more depressed pileus, brown colour, strongly decurrent lamellae, bigger spores ( $X_m = 10.2 \times 9.6$  μm), and differently-shaped cheilocystidia. *E. rusticoides* has a darker basidiomata, presents a more fibrillose surface of the pileus, is translucently striate in well-hydrated specimens, and lacks cystidia. *E. cettoi* Noordel., Hauskn. & Zuccherelli, sometimes presents a tomentose greyish pileus, but can be easily differentiated by its heterodiametrical spores, and the absence of incrusting pigment. Finally, *E. chelone* differs because of the absence of cheilocystidia and smaller spores, 6.5–7.5 × 5–6.5 μm.



**Fig. 6.** *Entoloma almeriense* (*typus*): a) spores; b) basidia; c) cheilocystidia. *E. phaeocarpum* (*typus*): d) spores; e) basidia. Scale bar = 8  $\mu$ m.





*Entoloma almeriense* (typus). Photo J. Carbó.



*Entoloma phaeocarpum* (typus). Photo A. Caballero.



*Entoloma rhodocylix* (K (M): 167653). Photo L. Goodwin.

7. *Entoloma phaeocarpum* F. Caball., Vila, A. Caball. & Català sp. nov.

Mycobank MB 807471

ETYMOLOGY. From greek *phaeo* = brown, and *carpos* = fructification.

TYPUS. SPAIN: Calahorra (la Rioja), alt. 400 m; among grasses and mosses, near *Pinus halepensis*, on sandy soil, 18 Oct. 2003, leg. A. Caballero, LIP JVG 1031018, *isotypus* in AC2706.

ORIGINAL DIAGNOSIS: Pileus up to 15 mm in diam., first flattened-convex, and somewhat depressed in the central disc, sometimes umbilicated, then flattened or slightly concave, neither hygrophanous nor translucently striate; surface velvety or slightly roughened; dark brownish, almost blackish in the center, with reddish tones near the margin; margin straight and somewhat cracked. Lamellae adnate to decurrent, distant, thick, broad; greyish with lilac shades or greyish-brown, turning pinkish with maturation; edge of the same colour, sinuose, irregular, with abundant veins on both sides; lamellulae present. Stipe central or eccentric, short, up to 10 × 3 mm, cylindrical, sometimes narrowed at the base, straight, with a smooth, glabrous surface; greyish-brown, with lilac shades somewhat lighter than those of the pileus. Flesh thin, of the same colour or lighter; smell faint, pleasant, slightly fruity, taste not tested. Spores isodiametrical, 8.6–10.2 × 7.8–9.8 μm, X<sub>m</sub> = 9.6 × 9.1 μm, Q = 1.01–1.19, Q<sub>m</sub> = 1.06, with an almost rounded perimeter and 5-7 weak angles. Basidia tetrasporic, claviform, with the neck much elongated from the base, 35–62.6 × 12–16.6 μm (without sterigmata), clampless. Cystidia absent. Pileipellis with a cutis structure composed of long cylindrical hyphae, constricted at the septa, up to 100 × 20 μm, mixed with thinner hyphae, up to 10 μm wide; mixed pigment, intraparietal and epiparietal incrustant, sometimes zebra-striped, present in all tissues examined. Clamp-connections not observed.

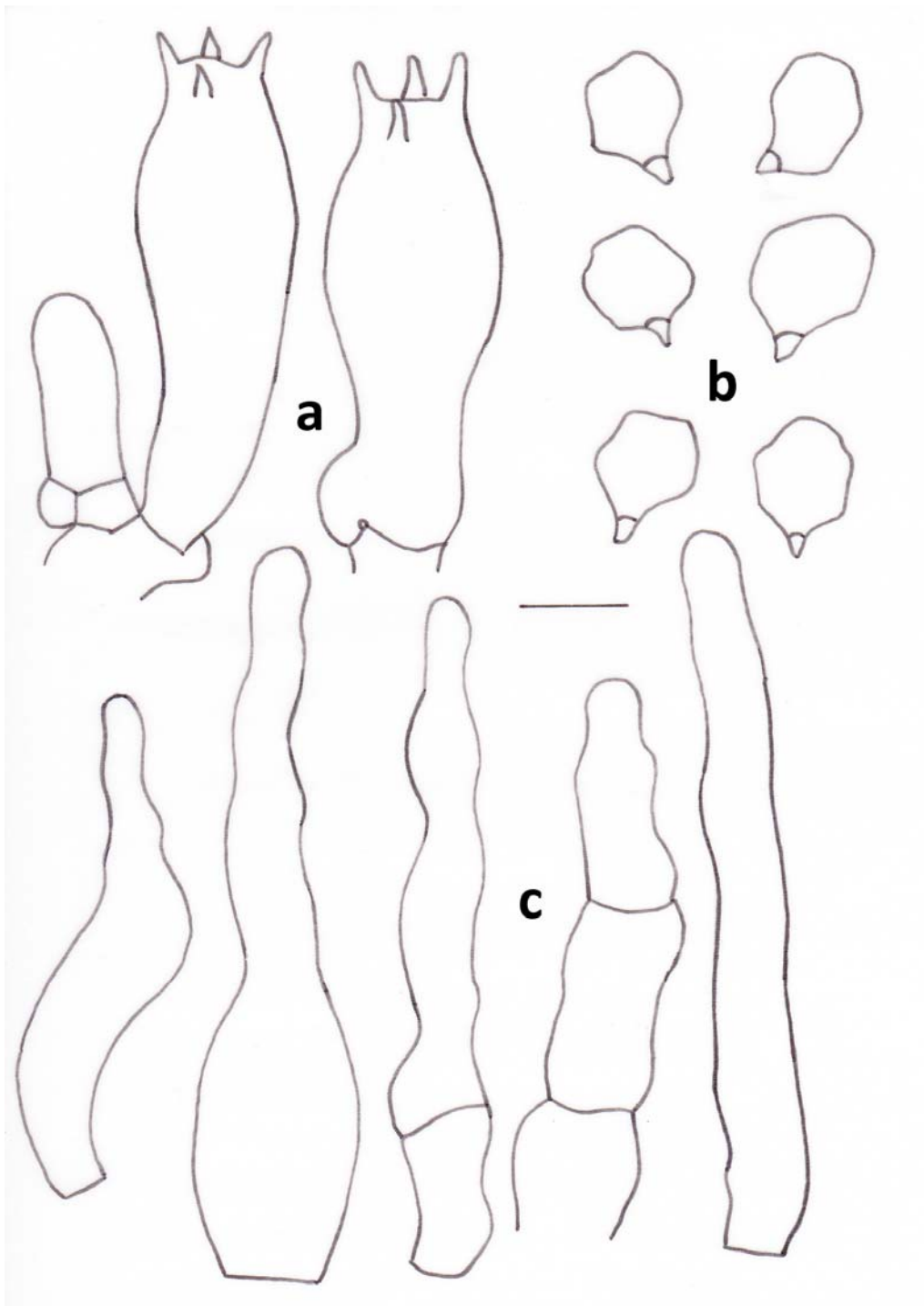
Because of its shape and habitat, *Entoloma phaeocarpum* can recall *E. rusticoides* or *E. phaeocyathus*, but it can be differentiated by the presence of intervenose lamellae, lilac tones in the lamellae and stipe, and by the often eccentric position of the latter, which can be very short. Microscopically, it shares the features of its group (large roundish spores, clamp-connections absent), but presents a more abundant intraparietal pigment in the pileipellis. Further collections will allow a better understanding of the variability of *E. phaeocarpum*.

8. *Entoloma* aff. *iodiolens* Arnolds & Noordel.

COLLECTION EXAMINED. SPAIN: Torrent de la Casassa, Espinavell (Girona), alt. 1400 m; under *Larix decidua* Mill. And *Pinus sylvestris* L., among mosses, 4 Aug. 2011, leg. O. Pérez-De-Gregorio and M.Á. Pérez-De-Gregorio, JC-20110804.1 (Ex-2335).

9. *Entoloma rhodocylix* (Lasch) M.M. Moser, Kleine Kryptogamenflora IIB/2 ed. 4: 210 (1978)

COLLECTIONS EXAMINED. UNITED KINGDOM: Moor Piece (England), among mosses and lichens, under *Picea* sp., 21 Sep. 2002, leg. T. Læssøe, K (M): 105174.- Hindhead (England), in sandy soil, under *Pinus* sp., 11 Oct. 2010, leg. L. Goodwin, K (M): 167653.- Mynydd Llwydiarth (Wales), among mosses, under conifers, 12 Sep. 2006, leg. C.E. Aron, K (M): 147598.



**Fig. 7.** *Entoloma rhodocylix* (K(M): 167653): a) basidia; b) spores; c) cheilocystidia. Scale bar = 8  $\mu$ m.



*Entoloma muscoalpinum* (typus). Photo J. Vila.



*Entoloma muscoalpinum* (paratypus). Photo J. Vila.

10. *Entoloma muscoalpinum* Vila, F. Caball. & Català sp. nov.= *E. lanicum* ss. Vila & Esteve-Raventós (1998)

MycoBank MB 807472

ETYMOLOGY. From latin *muscus* = moss and *alpinum* = living in the alpine belt.TYPUS. SPAIN: Vall de Núria, Queralbs (Girona), alt. 2220 m; among mosses (*Mnium spinulosum* Bruch & Schimp., *Distichium capillaceum* (Hedw.) Bruch & Schimp. and *Myurella julacea* (Swägr.) Schimp., det. I. Alvaro), in basic soil, 27 Aug. 1997, leg. J. Vila, LIP JVG 970827J.

ORIGINAL DIAGNOSIS: Pileus up to 15 mm in diam., convex to flattened-convex, somewhat depressed at the central disc in fully mature specimens, slightly hygrophanous, not translucently striate; surface densely covered with a pruinose-pubescent whitish layer, especially in young specimens, turning smooth or almost glabrous with age; below this layer, greyish when young, then whitish with yellowish or greyish stains; margin straight to somewhat curved. Lamellae adnate, frequently with a small decurrent tooth or clearly decurrent, distant, thick, dark grey with pinkish tones when spores are mature; edge of the same colour, entire or slightly irregular; lamellulae present. Stipe central, up to 20 × 4 mm, cylindrical, in some specimens with a small rooting base, straight or curved; greyish to pale brownish-grey, rarely with yellowish stains, covered with a pruinose or minutely pubescent whitish layer; pruinose apex, tomentose base. Flesh thin, whitish; smell radish-like, not intense; taste not tested. Spores heterodiametrical, 8.5–10 × 6–7.8 µm, with 5-7 well-marked angles. Basidia tetrasporic, or rarely bi- or monosporic, narrowly clavate, up to 40 × 8 µm, clamped. Cystidia not observed. Pileipellis with a cutis structure composed of narrow cylindrical hyphae, up to 6 µm wide, with slightly widened, cylindrical to claviform terminal elements, up to 8 µm wide; very diffuse pigment, epiparietal incrustant and intraparietal. Caulopellis with cylindrical to sinuose cells, sometimes pigmented in a similar way as the terminal elements of the pileipellis. Clamp-connections present in the hymenium, not observed in the pileipellis.

ADDITIONAL COLLECTIONS EXAMINED. SPAIN: Vall de Núria, Queralbs (Girona), alt. 2220 m; among mosses (*Mnium spinulosum*, *Distichium capillaceum* and *Myurella julacea*, det. I. Alvaro), in basic soil, 12 Aug. 1997, leg. J. Vila, JVG 970812-23.-*Ibid.*, 30 Aug. 2013, leg. J. Vila, JVG 1130830-11 (*paratypus*).

*Entoloma muscoalpinum* is not related to the *rusticoides* group, due to its heterodiametrical spores and clamped hyphae, but is indeed included in the present work because its macroscopical appearance can recall the species of this group. *E. muscoalpinum* is characterized by its (i) bicolour basidiomata, the pileus being always lighter than lamellae, (ii) yellow stains observed in some collections, (iii) densely pubescent pileus and stipe surface, more evident in young specimens, and (iv) alpine habitat, commonly being found among mosses, above 2000 masl. These features allow this species to be differentiated from *E. undatum* (Gillet) M.M. Moser or *E. lanicum* (Romagn.) Noordel. Not reliable phylogenetic data of *E. muscoalpinum* was obtained from the samples collected. Further analyses are needed to confirm the phylogenetic position of this species. For more information, including type-drawings, see VILA & ESTEVE-RAVENTÓS (1998, *sub E. lanicum*).

**Table 1.** Database GenBank/UNITE accession numbers for specimens included in the phylogenetic analyses.

Taxon	Voucher/Strain	ITS	28S nrLSU
<i>Entoloma almeriense</i>	LIP JVG 990105I TYPUS	KJ001428	
<i>Entoloma byssisedum</i> var. <i>byssisedum</i>	JVG 1080907-1	KJ001413	
<i>Entoloma byssisedum</i> var. <i>microsporium</i>	JVG 1061028-3	KJ001409	
<i>Entoloma flocculosum</i>	JVG 1080920-20	KJ001438	KJ001463
<i>Entoloma flocculosum</i>	Ludwig Nr. 2399 (Koll. C)	KJ001439	
<i>Entoloma flocculosum</i>	Ludwig Nr. 2399 (Koll. A)	KJ001440	
<i>Entoloma graphitipes</i> f. <i>graphitipes</i>	JVG 1121026-1	KJ001442	
<i>Entoloma graphitipes</i> f. <i>graphitipes</i>	EFC 11112006-83	KJ001444	KJ001460
<i>Entoloma graphitipes</i> f. <i>graphitipes</i>	Ludwig Nr. 3348 TYPUS	KJ001443	
<i>Entoloma graphitipes</i> f. <i>graphitipes</i>	AC1559	KJ001446	
<i>Entoloma graphitipes</i> f. <i>cystidiatum</i>	SFC 09010202	KJ001447	KJ001457
<i>Entoloma graphitipes</i> f. <i>graphitipes</i>	EFC 25112006-87	KJ001448	KJ001459
<i>Entoloma graphitipes</i> f. <i>graphitipes</i>	JVG 1071208-10	KJ001449	KJ001458
<i>Entoloma graphitipes</i> f. <i>cystidiatum</i>	LIP JVG 1090222G TYPUS	KJ001445	KJ001456
<i>Entoloma halophilum</i>	LIP JVG 961228H TYPUS	KJ001441	KJ001461
<i>Entoloma</i> aff. <i>iodiolens</i>	JC-20110804.1 (Ex-2335)	KJ001429	KJ001464

Taxon	Voucher/Strain	ITS	28S nrLSU
<i>Entoloma phaeocarpum</i>	LIP JVG 1031018 TYPUS La Rioja, Spain	KJ001430	KJ001462
<i>Entoloma phaeocyathus</i>	LIP JVG 960203 EPITYPUS Girona, Spain	KJ001418	KJ001468
<i>Entoloma phaeocyathus</i>	ACM 210169 València, Spain	KJ001417	KJ001467
<i>Entoloma phaeocyathus</i>	MES 2713 Alacant, Spain		KJ001476
<i>Entoloma phaeocyathus</i>	SFC 11020301-01 Barcelona, Spain	KJ001419	KJ001466
<i>Entoloma phaeocyathus</i>	MHR040608 Segovia, Spain	KJ001420	KJ001473
<i>Entoloma phaeocyathus</i>	MHR220508 Palencia, Spain	KJ001421	KJ001470
<i>Entoloma phaeocyathus</i>	JVG 1111118-3 Girona, Spain	KJ001424	KJ001474
<i>Entoloma phaeocyathus</i>	JVG 1111118-2 Girona, Spain	KJ001425	KJ001472
<i>Entoloma phaeocyathus</i>	JVG 1111118-4 Girona, Spain	KJ001426	KJ001475
<i>Entoloma phaeocyathus</i>	JC-20060204.2 (Ex-1859) Girona, Spain	KJ001422	KJ001469
<i>Entoloma phaeocyathus</i>	JC-20070218.1 (Ex-1932) Girona, Spain	KJ001423	KJ001471
<i>Entoloma phaeocyathus</i>	JVG 1111119-2 Barcelona, Spain	KJ001427	KJ001465
<i>Entoloma rhodocylix</i>	K (M): 167653 England, United Kingdom	KJ001414	KJ001452
<i>Entoloma rhodocylix</i>	K (M): 147598 Wales, United Kingdom	KJ001415	KJ001450
<i>Entoloma rhodocylix</i>	K (M): 105174 England, United Kingdom	KJ001416	KJ001451
<i>Entoloma rusticoides</i>	JC-20031124.8 (Ex-1656) Mallorca, Spain	KJ001432	KJ001479



Taxon	Voucher/Strain	ITS	28S nrLSU
<i>Entoloma rusticoides</i>	JVG 941025-2	Lleida, Spain	KJ001483
<i>Entoloma rusticoides</i>	JVG 941025-1	Lleida, Spain	KJ001433
<i>Entoloma rusticoides</i>	LIP JVG 1020416U EPITYPUS	Tarragona, Spain	KJ001434
<i>Entoloma rusticoides</i>	SCG-231011-3	València, Spain	KJ001435
<i>Entoloma rusticoides</i>	SFC 1107171	Barcelona, Spain	KJ001437
<i>Entoloma rusticoides</i>	SCG-0411111-1	València, Spain	KJ001436
<i>Entoloma rusticoides</i>	JVG 1081105-12	Tarragona, Spain	KJ001484
<i>Entoloma rusticoides</i>	JC-20121104.1 (Ex-2474)	Girona, Spain	KJ001431
<i>Entoloma undatum</i>	JVG 1051115-19	Girona, Spain	KJ001410
<i>Entoloma undatum</i>	JVG 1060830-8	Lleida, Spain	KJ001411
<i>Entoloma undatum</i>	JVG 1111118-5	Girona, Spain	KJ001412
<i>Entoloma undulatosporum</i>	SFC 11021902	Barcelona, Spain	KJ001412
GenBank complementary sequences			
<i>Entoloma abortivum</i>			JQ281483
<i>Entoloma abortivum</i>			GQ166906
<i>Entoloma abortivum</i>			JQ320131

Taxon	Voucher/Strain	ITS	28S nrLSU
<i>Entoloma abortivum</i>		GQ289150	
<i>Entoloma abortivum</i>		AF223169	
<i>Entoloma indoviolaceum</i>		GQ289172	
<i>Entoloma parasiticum</i>		GQ289177	
<i>Entoloma sericeum</i>		GQ289191	
<i>Entoloma tjallingiorum</i>		GQ289197	
<i>Entoloma undatum</i>		AF261314	
<i>Entoloma undatum</i>		AY207199	
<i>Entoloma vezzenaense</i>		GQ289204	
Uncultured basidiomycete clone 4M1_E06		EU489915	
Uncultured fungus clone MM209		JX316693	
UNITE complementary sequences			
<i>Entoloma korhonenii</i>		UDB011454	
<i>Entoloma korhonenii</i>		UDB017915	

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## REFERENCES

- BRESADOLA, G. in SACCARDO, P.A. (1891).- *Sylloge fungorum omnium hucusque cognitorum*.
- CABALLERO, F. & J. VILA (2013).- *Entoloma* nuevos o interesantes de la Península Ibérica (3). Adiciones y correcciones. *Fungi non Delineati* LXVI (*Studies on Entoloma*): 63-85, 136-145 (iconografía). Ed. Candusso, Alassio.
- CO-DAVID, D.; LANGEVELD, D. & M.E. NOORDELOOS (2009).- Molecular phylogeny and spore evolution of *Entolomataceae*. *Persoonia* 23: 147–176.
- COURTECUISSÉ, R. (1993).- Macromycètes intéressants, rares ou nouveaux (VI). *Entolomataceae. Documents mycol.* 89: 1–38.
- GARDES, M. & T.D. BRUNS (1993).- ITS primers with enhanced specificity for basidiomycetes. Application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113-118.
- GILLET, C.C. (1876).- *Les Hyménomycètes ou description de tous les champignons qui croissent en France*. pp. 177-560.
- HE, X.L.; LI, T.H.; XI, P.G.; JIANG, Z.D. & Y.H. SHEN (2013).- Phylogeny of *Entoloma* s.l. subgenus *Pouzarella*, with descriptions of five new species from China. *Fungal Diversity* 58(1): 227–243.
- LUDWIG, E. (2007).- Pilzkompodium. Band 2. Die größeren Gattungen der Agaricales mit farbigem Sporenpulver (ausgenommen Cortinariaceae). Beschreibungen (723 pp.) + Abbildungen (209 pp.). Fungicon-Verlag, Berlin.
- MORGADO, L.N.; NOORDELOOS, M.E.; LAMOUREUX, Y. & J. GEML (2013).- Multi-gene phylogenetic analyses reveal species limits, phylogeographic patterns, and evolutionary histories of key morphological traits in *Entoloma* (*Agaricales, Basidiomycota*). *Persoonia* 31: 159-178.
- MOROZOVA, O.V.; NOORDELOOS, M.E. & J. VILA (2014).- *Entoloma* subgenus *Leptonia* in boreal-temperate Eurasia: towards a phylogenetic species concept. *Persoonia* 32 (in press).
- NOORDELOOS, M.E. (1992).- *Entoloma* s.l. *Fungi Europaei* 5, Libreria editrice G. Biella, Saronno. 760 pp.
- NOORDELOOS, M.E. (2004).- *Entoloma* s.l. *Fungi Europaei* 5A, Edizioni Candusso, Alassio. 618 pp.
- NYLANDER, J.A.A. (2004).- MrModeltest v2. Program distributed by the author. Uppsala, Evolutionary Biology Centre, Uppsala University.
- RONQUIST, F. & J.P. HUELSENBECK (2003).- MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.
- STAMATAKIS, A. (2006).- RAXML-VI-HPC: maximum likelihoodbased phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.
- SWOFFORD, D.L. (2001).- PAUP\*4.0b10: phylogenetic analysis using parsimony (and other methods). Sunderland, Sinauer Associates.
- TAMURA, K.; PETERSON, D.; PETERSON, N.; STECHER, G.; NEI, M. & S. KUMAR (2011).- MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28 (10): 2731-2739.
- VILA, J. & F. CABALLERO (2007).- *Entoloma* nuevos o interesantes de la Península Ibérica. *Fungi non Delineati* XXXVIII. 64 pp. Ed. Candusso, Alassio.
- VILA, J. & F. CABALLERO (2009).- *Entoloma* nuevos o interesantes de la Península Ibérica. II. *Fungi non Delineati* XLV. 100 pp. Ed. Candusso, Alassio.

- VILA, J. & F. ESTEVE-RAVENTÓS (1998).- *Dermoloma pseudocuneifolium*, *Entoloma lanicum* y *E. scabropellis* (*Basidiomycetes*) en el piso alpino del Valle de Núria (Pirineos, Catalunya). *Bol. Soc. Micol. Madrid*, 23: 151-158.
- VILA, J.; CARBÓ, J.; CABALLERO, F.; CATALÀ, S.; LLIMONA, X. & M.E. NOORDELOOS (2013).- A first approach to the study of the genus *Entoloma* subgenus *Nolanea* s.l. using molecular and morphological data. *Fungi non Delineati LXVI (Studies on Entoloma)*: 3-62, 93-135 (iconography). Edizioni Candusso, Alassio.
- VILGALYS, R. & M. HESTER (1990).- Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* 172: 4238-4246.
- WHITE, T.J.; BRUNS, T.; LEE, S. & J. TAYLOR (1990).- *Amplification and direct sequencing of fungal ribosomal DNA genes for phylogenetics*. In M. INNIS, J. GELFAND, J. SNINSKY & T. WHITE. PCR protocols: A guide to methods and applications: 315-322, Academic Press, Orlando, Florida.