

A new *Leucoagaricus* species of section *Piloselli* (Agaricales, Agaricaceae) from Spain

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Abstract: The new species *Leucoagaricus variicolor* is described from a public park in Zaragoza, Spain, based on both morphological and molecular characters. Illustrations of fresh basidiomata *in situ* and of the main macro- and micromorphological features are added. *Leucoagaricus variicolor* belongs to section *Piloselli* and is compared with similar taxa.

Key words:

Agaricomycetes
Basidiomycota
nrITS rDNA
taxonomy

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INTRODUCTION

During a survey of macrofungi conducted by the first author in a public park in Zaragoza (Parque José Antonio Labordeta), four collections of a remarkable species of *Leucoagaricus* were recorded on clayey soil near a *Pinus halepensis* plantation. The park covers an area of 409 000 sq. m to the south of the city; it is regarded as an important green space because of its botanical biodiversity. The species has been collected from an area of 50 sq. m.

The collections fit morphologically into *Leucoagaricus* sect. *Piloselli*, a section within the *Leucoagaricus/Leucocoprinus* clade (*Agaricaceae*) that encompasses species whose basidiomata usually stain orange-red when bruised and turn green with ammonia (Singer 1973, 1986, Vellinga 2010). In this section, species identification depends particularly on morphological characters such as pileus colour, colour reactions of the basidiome surface when bruised or exposed to ammonia, the structure of the pileipellis, and the shape of the cheilocystidia and spores (Bon 1993, Vellinga 2010). Species of the morphologically similar sect. *Rubrotincti* differ mainly in the immutable context (not changing colour when bruised) and the absence of a green reaction with ammonia on the basidiome surface (Singer 1948, 1986, Bon 1993, Vellinga 2001).

Our taxon is distinguished from all other species in sect. *Piloselli* by the cream-ochre pileus which becomes entirely pink-rose in herbarium specimens, abundant velar remnants on the pileus surface, mainly pyriform to sphaeropedunculate cheilocystidia, and subglobose to broadly ellipsoid spores. An exhaustive search of the literature, including monographic

treatments and papers by Bon (1981, 1993), Candusso & Lanzoni (1990), Contu (1990), Bon & Caballero (1997), Caballero (1997), Gennari & Migliozi (1999), Migliozi & Resta (2001), Migliozi *et al.* (2001), Vellinga (2001, 2006, 2010), and Vellinga *et al.* (2010), confirmed the unique nature of this species: its characteristics do not match any published species. In addition, an ITS sequence analysis supported this statement. Therefore, a detailed description and illustrations of this previously undescribed *Leucoagaricus* are presented here.

MATERIALS AND METHODS

Morphology

All the studied collections were photographed *in situ*, using a Nikon D50 digital camera, with a tripod, and in natural light. Macromorphological features are described from fresh specimens. The microscopic structures were observed in both fresh and dried material, using several mountants and stains: water, 2 % KOH, ammoniacal Congo red, Brilliant Cresyl blue, and Melzer's reagent. Dried fragments were rehydrated in 2 % KOH. All microscopic measurements were carried out with a ×1000 oil immersion objective. In the description below, spore measurements are based on 120 elements in ammoniacal Congo red randomly selected from four collections. Only mature, normally developed and non-aberrant spores from spore prints were measured. Dimensions of the spores are given as follows: (minimum value–) 1st decile – average value – 9th decile (–maximum value). The width of basidia was measured at the widest part, and the length was measured from the apex (sterigmata

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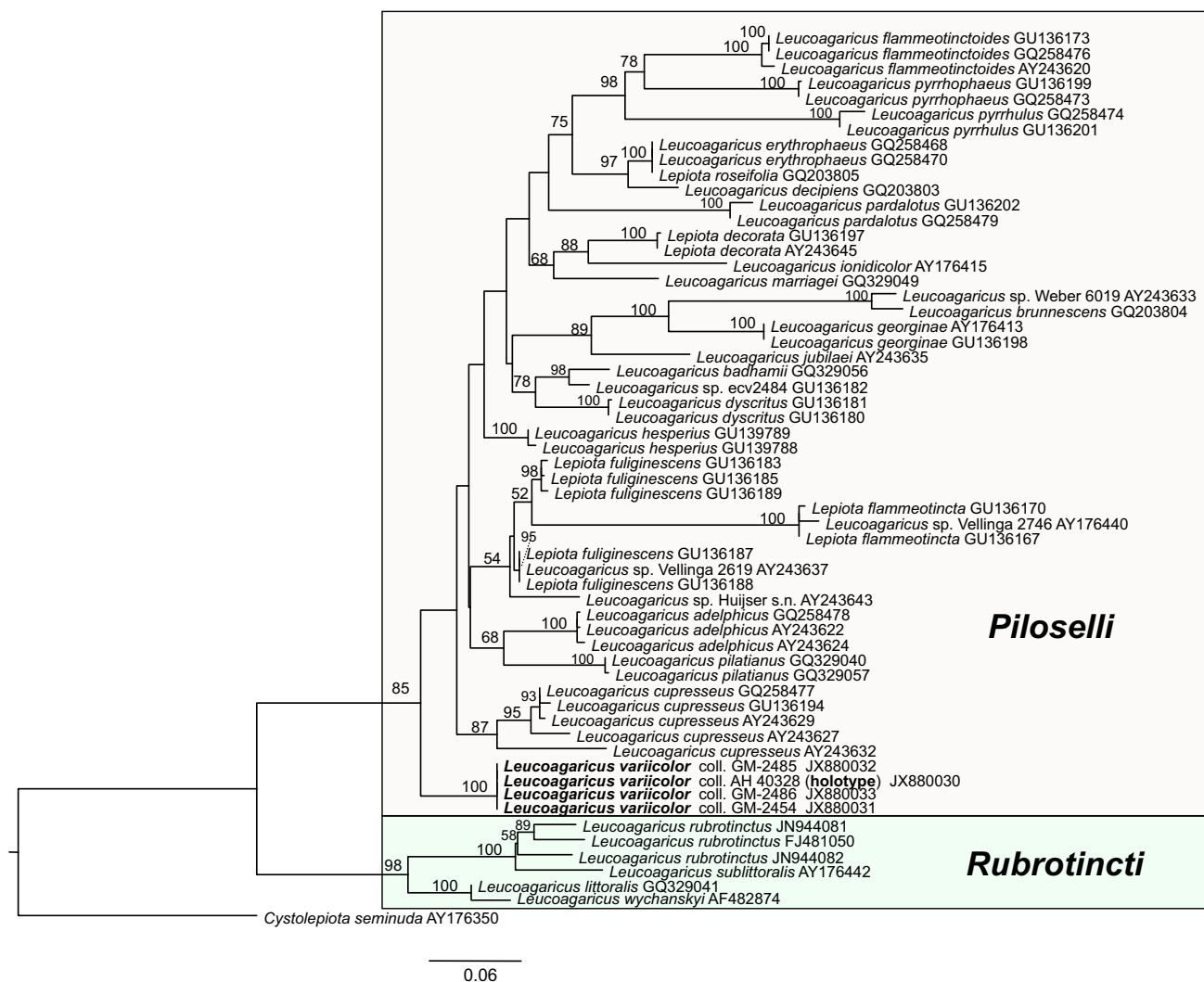


Fig. 1. Maximum Likelihood phylogram obtained from the ITS (ITS1-5.8S-ITS2) sequence alignment of *Leucoagaricus* spp. *Cystolepiota seminuda* was used as outgroup taxon. MLB values over 50 % are given above branches. Newly sequenced collections are in bold.

excluded) to the basal septum. Microscopic pictures were taken on a Moticam 2500 digital camera connected to a Motic BA300 microscope. Colour notations for the macroscopic descriptions are from Munsell (1994), hereafter shortened as Mu. Herbarium acronyms follow *Index Herbariorum*, except for GM and AC that refer to the personal herbaria of Guillermo Muñoz and Agustín Caballero. The type collection is housed at AH. The name and description of the new species are deposited in MycoBank (Crous *et al.* 2004).

DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was isolated from 1 mg of a dried herbarium specimen from four collections (AH-40328, GM-2454, GM-2485, and GM-2486), using the DNeasy Plant Mini Kit (Qiagen, Milan) according to the manufacturer's instructions. Universal primers ITS1F/ITS4 were used for the ITS region amplification (White *et al.* 1990, Gardes & Bruns 1993). Amplification reactions were performed in a PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) following Vizzini *et al.* (2011). The PCR products were purified with the AMPure

XP kit (Beckman) and sequenced by MACROGEN (Seoul, Republic of Korea). The sequences were submitted to GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) and their accession numbers are reported in Fig. 1.

Sequence alignment and phylogenetic analysis

The sequences obtained in this study were checked and assembled using Geneious v. 5.3 (Drummond *et al.* 2010) and compared to those available in the GenBank database by using the Blastn algorithm. Based on the Blastn results, sequences were selected according to the outcomes of recent phylogenetic studies on *Leucoagaricus* (Vellinga 2010, Vellinga *et al.* 2010, 2011). Alignments were generated using MUSCLE (Edgar 2004) with default conditions for gap openings and gap extension penalties. The alignment was then imported into MEGA v. 5.0 (Tamura *et al.* 2011) for manual adjustment. The phylogenetic analysis was performed using the Maximum Likelihood (ML) approach. Following Vellinga (2010) and Vellinga *et al.* (2010), a *Cystolepiota seminuda* sequence (AY176350) was used as outgroup. ML estimation

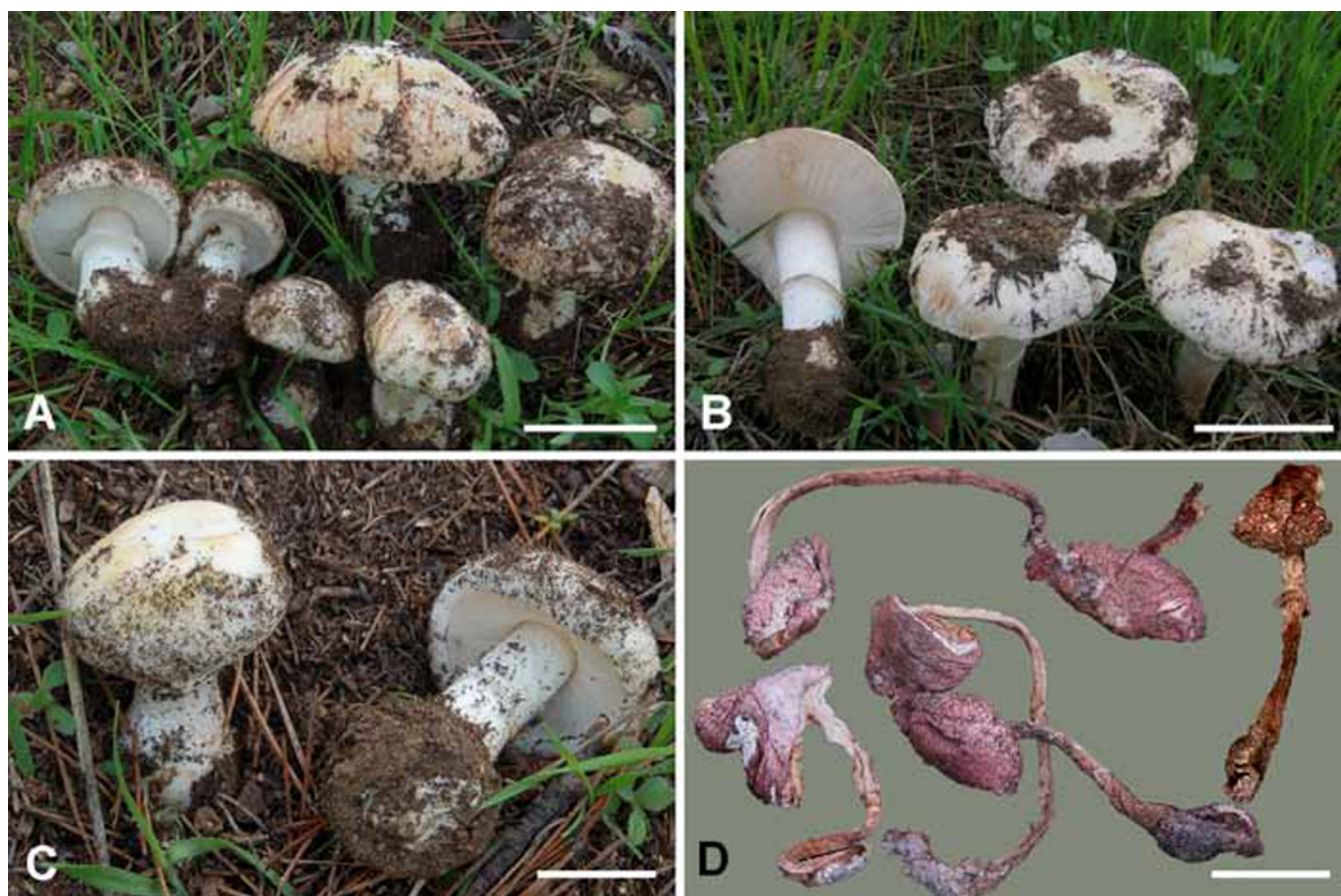


Fig. 2. *Leucoagaricus variicolor*. Macroscopic characters. **A–C.** Fresh basidiomata *in situ*. **D.** Herbarium specimens. A, D from AH 40328 (holotype); B from GM-2454; C from GM-2485. Bars = A–B = 50 mm; C–D = 20 mm.

was performed through RAxML v. 7.0.4 (Stamatakis 2006) with 1000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMA algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the “-f a” option of RAxML and “-x 12345” as a random seed to invoke the novel rapid bootstrapping algorithm. Only MLB over 50 % are reported in the resulting tree (Fig. 1).

RESULTS

Phylogenetic analysis

The amplification of the ITS regions was successful for the four specimens, yielding a PCR product of about 700 bp. The ITS data matrix comprises a total of 59 sequences (including 55 from GenBank). In the obtained ML phylogram (Fig. 1), our four sequences of the new *Leucoagaricus* clustered together and are distinct and basal to all the existing sequences of previously sequenced species of section *Piloselli*.

Taxonomy

Leucoagaricus variicolor G. Muñoz, A. Caball., Contu & Vizzini, *sp. nov.*
Mycobank MB801565
(Figs 2–4)

Etymology: The specific epithet “*variicolor*” refers to the highly variable colours of the pileus surface depending on fresh or dry conditions.

Diagnosis: Differs from every other described species of *Leucoagaricus* sect. *Piloselli* in having a cream to pink to egg-yellow, or ochre pileus in fresh basidiomata that becomes dark rose in the herbarium, the presence of universal veil remnants on the pileus surface and stipe base, subglobose to broadly ellipsoid spores, pyriform to spheropedunculate cheilocystidia, and a unique ITS sequence.

Type: **Spain:** Aragón: Zaragoza, “José Antonio Labordeta” (*antea*: “Parque Grande”) park, UTM 30TXM7511, N 41.634 W 0.894, alt. 225 m, on argillose-sandy soil, in *Pinus halepensis* litter, 3 Dec. 2011, G. Muñoz (AH 40328 – **holotype**; GM-2453 and AC-4896 – **isotypes**).

Description: *Pileus* 40–100 mm wide, when young hemispherical-convex to hemispherical, expanding to subplane, finally plane, without an umbo, with an entire, slightly exceeding margin, involute or incurved in young stages; pileus surface dry, almost smooth to finely felted-fibrillose, variable in colour, starting from whitish or pale cream or egg-yellow to ochre (Mu 2.5Y 8/1-2; Mu 5Y 8/1 “White”; Mu 2.5Y 8/2-4 “Pale yellow”) to orange-pink (Mu 5YR 6/6-8 “Yellowish red”; Mu 2.5YR 5/6-8 “Red”); in adult stages

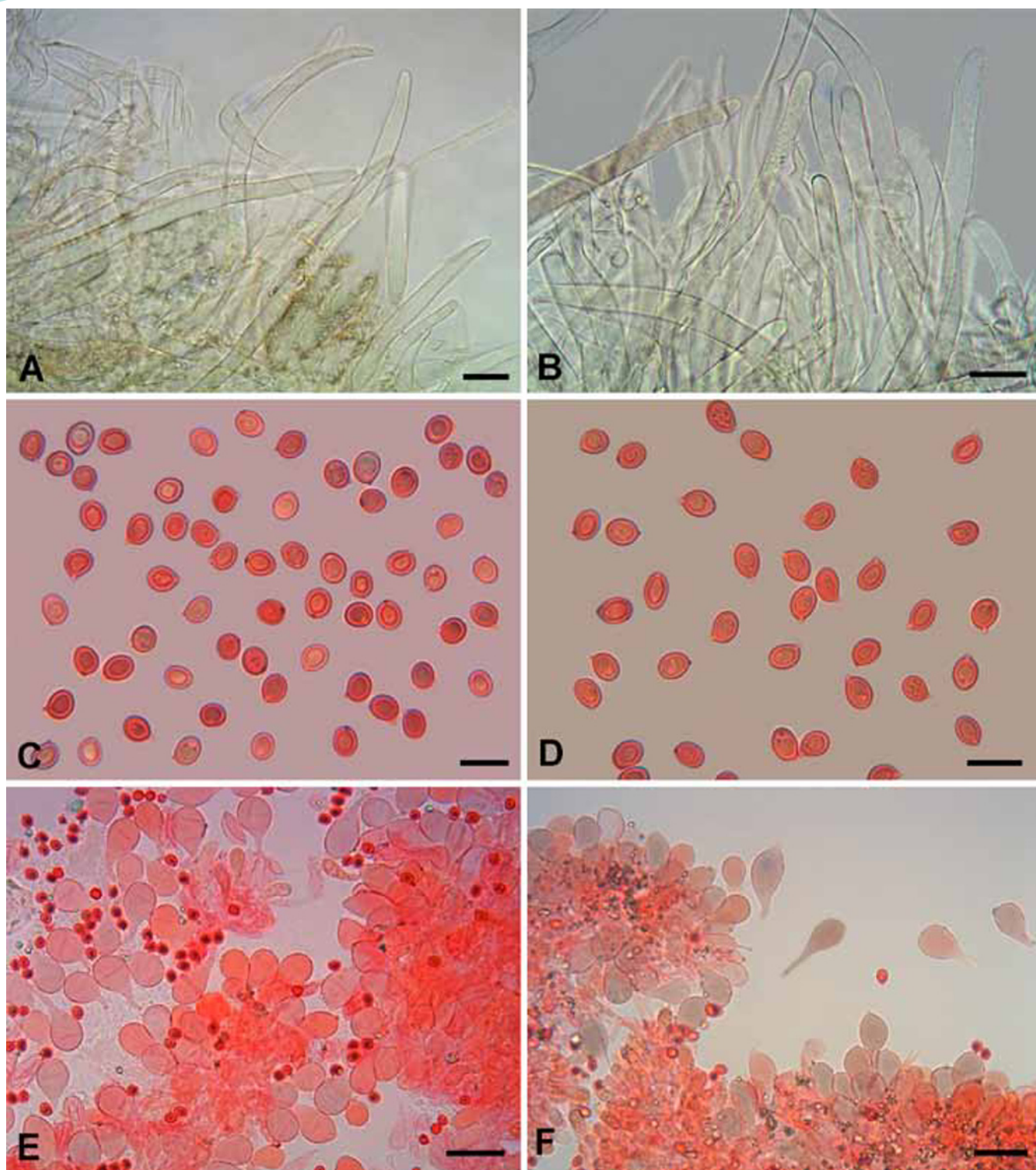


Fig. 3. *Leucoagaricus variicolor*. Microscopic characters. **A–B.** Elements of the pileipellis. **C–D.** Spores (in ammoniacal Congo red). **E–F.** Cheilocystidia (in ammoniacal Congo red). A, C, E from AH 40328 (holotype); B, D, F from GM-2485. Bars: A–B, E–F = 20 μ m; C–D = 10 μ m.

these tinges are mixed, in dried herbarium material the main tinge is pink-rose (Mu 10R 5/3-4; 6/3-4) (Fig. 2D); surface not reddening but tardily darkening on handling, covered, mainly toward the disc, but often up to the antimarginal zone, with abundant, fibrillose to submembranaceous white velar patches, as remnants of the universal veil. *Lamellae* not reaching the stipe, attached to a pseudocollarium, crowded, to 6 mm broad, with (0–)1–3 lamellulae, white to cream or

slightly beige, darker towards the margin, not reddening when bruised, with an even or slightly flocculose, concolorous edge. *Stipe* 40–100 \times 12–18 mm, stout, solid, cylindrical, in most specimens with a napiform to submarginate bulb and then up to 23 mm wide; surface white, shiny, pruinose-floccose at the apex, at times slightly browning on handling or due to the environmental conditions, in young stages with minute universal veil remnants (as coarse flecks) towards the base.

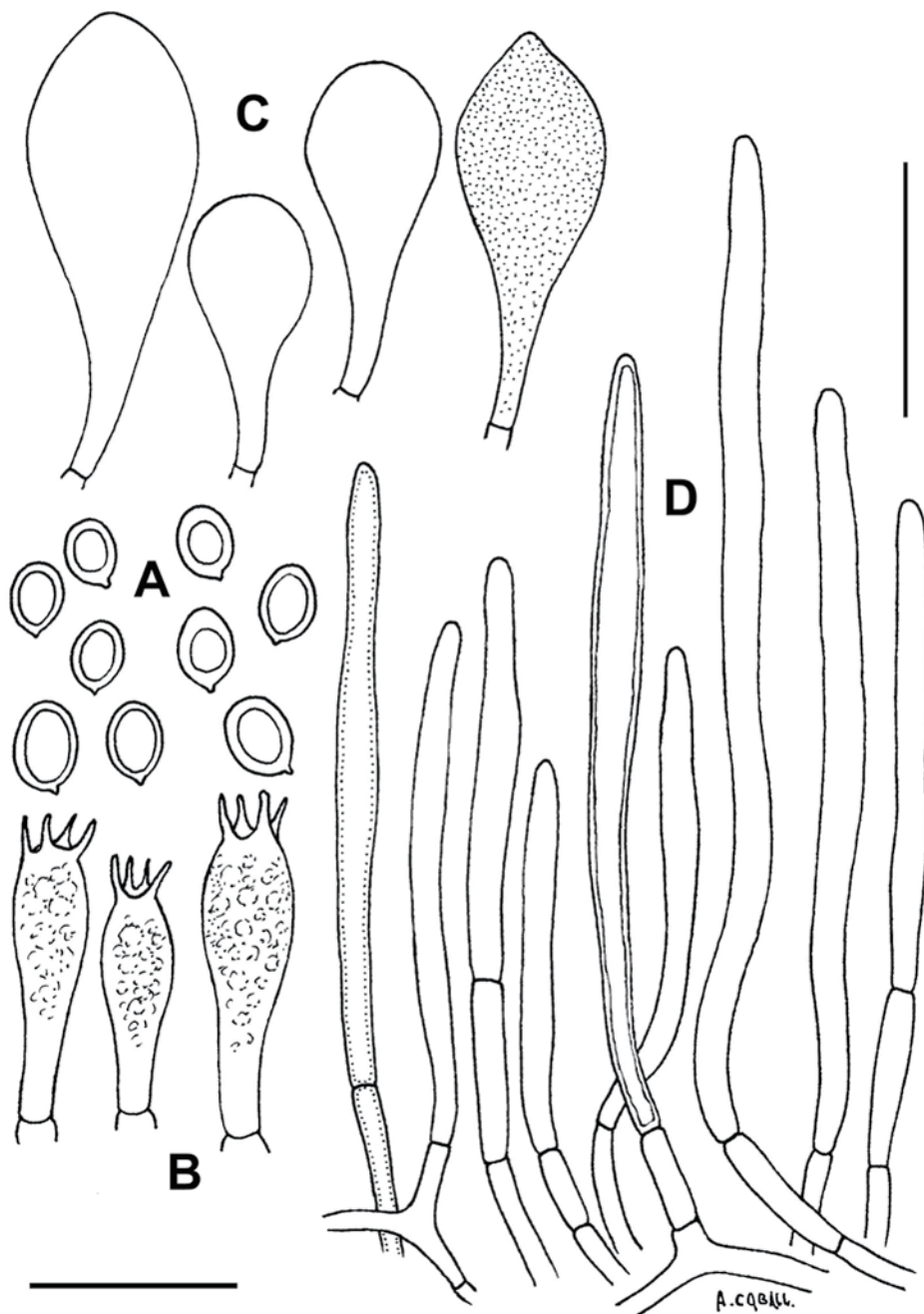


Fig. 4. *Leucoagaricus variicolor*. Line drawings of microscopic characters (from AH 40328, holotype). **A.** Spores. **B.** Basidia. **C.** Cheilocystidia. **D.** Pileipellis. Bars: A–C = 20 µm; D = 100 µm.

Annulus thin, simple, membranous, persistent, not movable, usually descending (rarely ascending), entirely white or ochre towards margin. *Context* fleshy, white, unchanging or slightly turning ochre-pink towards the base of the stipe. *Smell* and taste not distinctive, fungoid, pleasant. *Edibility* unknown. *Spore-print*: white. *Chemical reactions*: surface of the pileus, stipe, annulus, and lamellae green in ammonia. *Spores* (5.6–)6.0–6.8–7.6 (–8.2) × (4.5–)4.8–5.2–5.6 (–5.7) µm, $Q = (1.1–)1.2–1.3–1.4 (–1.6)$ ($n = 120$), subglobose to broadly ellipsoid or slightly ovoid, smooth, without a germ-pore, dextrinoid, metachromatic in Cresyl Blue (Figs 3C, D, 4A). *Basidia* 20–30 × 7–9 µm four-spored, clavate, without basal clamp connection (Fig. 4B). *Lamella edge* sterile. *Cheilocystidia* abundant, 20–45 × 10–16 µm, pedicellate, pyriform to sphaeropedunculate, rarely with a very short mucro, nearly hyaline or with light brown contents (diluted and homogeneous cytoplasmic pigment) (Figs 3E, F, 4C).

Pleurocystidia absent. *Pileipellis* a trichoderm consisting of erect, long cylindrical to fusiform, not gelatinized elements, occasionally septate, 150–350(–400) × 8–16 µm, without a subtending (basal) hymeniform layer (Figs 3A, B, 4D); pigment brownish, usually parietal, smooth, but sometimes also intracellular in some terminal elements. *Velar patches* of the pileus surface composed of hyaline, tightly interwoven, cylindrical, 3–7 µm wide hyphae. *Clamp-connections* absent.

Habitat and distribution: Terrestrial, on clayey soil, in the litter of a *Pinus halepensis* plantation, in a park with considerable public pressure. Basidiomes produced in winter (December). Known only from the province of Aragón, Spain, at this time.

Additional collections examined: **Spain**: Aragón: Zaragoza, “José Antonio Labordeta” (antea: “Parque Grande”) park, N 41.634 W 0.894, alt. 225 m, near *Pinus halepensis*, on argillous-sandy soil,

basidiomata nearly covered by the substrate, 3 Dec. 2011, G. Muñoz (GM-2454); *ibid.*, in closeby neighbourhoods, on soil, 10 Dec. 2011, G. Muñoz (GM-2485, GM-2486).

DISCUSSION

According to morphological data and phylogenetic analyses of ITS sequences (Fig. 1), the collections studied merit recognition as an independent species within *Leucoagaricus* sect. *Piloselli*. No similar species could be found in the literature since all the previously described species are distinguished by different tinges in the pileus, the absence of pyriform to spheropedunculate cheilocystidia, or more elongated and differently shaped spores (Candusso & Lanzoni 1990, Bon 1993, Caballero 1997, Gennari & Migliozi 1999, Migliozi & Resta 2001, Migliozi *et al.* 2001, Vellinga 2001, 2006, 2010).

Among the macromorphologically most similar species, *Lepiota decorata* (*Leucoagaricus idae-fragum* fide Vellinga 2006), known from the western parts of North America (California and Oregon) and the western parts of France (Atlantic coast), differs in the rose-vinaceous purple, raspberry deep pink overall colours from the first, narrowly clavate, cylindrical, to slightly utriform cheilocystidia, and ellipsoid to amygdaliform spores (Guinberteau *et al.* 1998, Vellinga 2006). *Leucoagaricus cupresseus*, known from under *Cupressaceae* in California, and the Atlantic and Mediterranean coasts of France, differs in the ellipsoid to oblong, amygdaliform spores with a faint papilla, and variably sized and shaped cheilocystidia (clavate, fusiform-clavate, lageniform-utriform, to cylindrical) (Sundberg 1976, Boisselet & Guinberteau 2001, Vellinga 2010). Finally, *L. pseudopilatianus* and its varieties, which occur in Spain and Italy, is distinguished by the red-brownish pileus, amygdaliform spores with an indistinct apical papilla, broader clavate cheilocystidia with evident brownish contents, a pileipellis with a subhymeniform basal layer, terminal elements of the pileipellis with rounded (not attenuated) tips, and basidiomes turning black on drying (Migliozi & Resta 2001, Migliozi *et al.* 2001); according to Vellinga (2010), that species could prove to be identical to *L. cupresseus*.

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