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

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Abstract

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

Keywords

Agaricomycetes *Basidiomycota* ITS sequences Molecular phylogeny Taxonomy

Introduction

Over the last 30 years, several specialists have made significant contributions to our understanding of the European species of the genus *Leucoagaricus* Locq. ex Singer. These include general monographic treatments (Bon [1981](#), [1993](#); Candusso and Lanzoni [1990](#)) and local floristic and taxonomic studies (Bon and Caballero [1997](#); Caballero [1997](#); Vellinga [2001](#)).

In *Leucoagaricus*, the species within section *Piloselli* Singer, typified by *Lepiota georginae* (W.G. Sm.) Sacc., are traditionally distinguished by basidiomes usually staining orange-red when bruised and turning green with ammonia and spores without germ pore (Singer [1973](#), [1986](#); Bon [1993](#); Vellinga [2010](#)). Species that show the same colour change but having spores with germ pore [e.g. *L. americanus* (Peck) Vellinga = *L. bresadolae* (Schulzer) Bon] have been included in sect. *Annulosi* (Fr.) Singer, typified by *L. leucothites* (Vittad.) Wasser (Singer [1973](#), [1986](#); Candusso and Lanzoni [1990](#); Bon [1993](#); Vellinga [2010](#)), a species with unchanging context and no reaction to ammonia; those that turn red, but not green, with ammonia, such as *L. croceovelutinus* (Bon & Boiffard) Bon & Boiffard, have also been accommodated in sect. *Piloselli* by some authors (e.g. Candusso and Lanzoni [1990](#); Bon [1993](#)).

Phylogenetic analyses of multiple loci (nrLSU, nrITS, *tef1* and *rpb2* regions; Vellinga [2004a, b](#); Vellinga and Sundberg [2008](#); Vellinga et al. [2011](#)) have pointed out that the species that redden with ammonia, those that turn green with ammonia with spores without a germ pore (sect. *Piloselli*), and those that turn green and have spores with a germ pore (sect. *Annulati* pro parte) do not form a monophyletic group. In particular, sect. *Piloselli* is polyphyletic even though most of them form a monophyletic cluster (named *Leucoagaricus* clade 3 in Vellinga et al. [2011](#)) including *L. macrorhizus* Locq. ex E. Horak (= *Lepiota barssii* Zeller according to Vellinga [2000](#)), the type species of *Leucoagaricus*.

The species of the morphologically allied sect. *Rubrotincti* Singer (monophyletic and named *Leucoagaricus* clade 1 in Vellinga et al. [2011](#)) differ mainly in the immutable context and the absence of a colour reaction with ammonia on the basidiome surface (Singer [1948, 1986](#); Bon [1993](#); Vellinga [2001](#)).

During a study on the *Agaricaceae* of the public park "José Antonio Labordeta" in Zaragoza (Aragón, Spain), the first two authors made four collections of a large, well-characterised *Leucoagaricus* species which proved impossible to identify. The basidiomes were collected on partially sandy soil at the margin of a small river among *Populus nigra*, *Populus alba*, *Fraxinus excelsior* and *Salix* sp. As the basidiome surfaces turn green with ammonia and the spores are without a germ pore, the collections belong to the section *Piloselli* but, as no name is available for it, we will introduce a new species based both on morphological and molecular data (ITS sequences analysis). From the macromorphological point of view, the new species is characterised by the saffron stipe base which turns rusty-brown with age in all the collected basidiomes. The main micromorphological features are the strictly clavate, not appendiculate and not pigmented cheilocystidia, the broadly ellipsoid to ellipsoid spores and the pileus surface consisting of a dry trichoderm of elongate, mainly fusiform hyphae with some shorter basal elements which are clavate or subfusiform.

The new taxon, which will be named *Leucoagaricus croceobasis* due to its saffron stipe base, is the second new species of *Leucoagaricus* discovered in the public park "José Antonio Labordeta" of Zaragoza, after *L. variicolor* G. Muñoz et al. (Muñoz et al. [2012](#)).

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. Colour notations for the macroscopic descriptions are from Munsell ([1994](#)), hereafter shortened as Mu. The microscopic structures were observed in both fresh and dried material, separately mounted in 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 $\times 1,000$ oil immersion objective. In the description below, spore measurements are based on 153 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 excluded) to the basal septum. Microscopic pictures were taken on a Moticam 2500 digital camera connected to a Motic BA300 microscope. The following abbreviations are used: L = number of entire lamellae; l = number of lamellulae between each pair of entire lamellae; Q = the

quotient of length and width of the spores in side view. The terminology used for describing the morphological characters referred to Vellinga (1998).

Herbarium acronyms follow *Index Herbariorum* (Thiers 2013), 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-40434, GM-2609, GM-2645 and GM-2732), using the DNeasy Plant Mini Kit (Qiagen, Milan, Italy) according to the manufacturer's instructions. Universal primers ITS1F/ITS4 were used for the ITS region amplification (White et al. 1990; Gardes and 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.

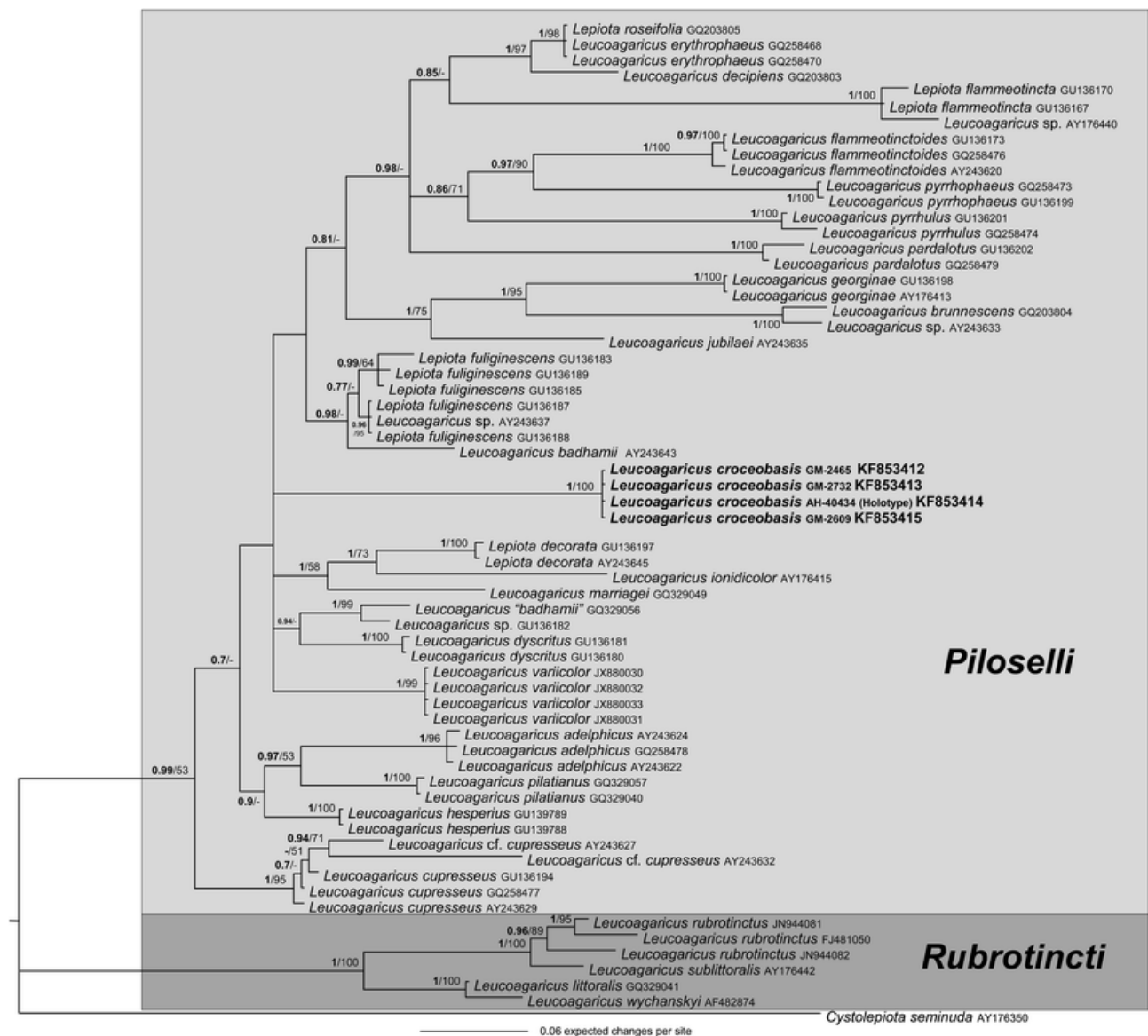


Fig. 1

Bayesian phylogram obtained from the general nrITS (ITS1-5.8S-ITS2) sequence alignment of *Leucoagaricus* spp. *Cystolepiota seminuda* was used as outgroup taxon. Only BPP values over 0.70 (in *bold*) and MLB values over 50 % are given above clade branches. Newly sequenced collections are in *bold*

Sequence alignment, dataset assembly 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 based on the greatest similarity) and outcomes of recent phylogenetic studies focused on sect. *Piloselli* (Vellinga [2004a](#), [b](#), [2010](#); Vellinga et al. [2010](#), [2011](#); Muñoz et al. [2012](#)), sequences were retrieved from GenBank for the comparative phylogenetic analysis. In this dataset, besides *Leucoagaricus* taxa that turn green with ammonia and have spores without germ pore, species representative of sect. *Rubrotincti* were chosen for comparison.

The alignment was generated using MAFFT (Katoh et al. [2002](#)) 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. Best-fit models were estimated by both the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with jModelTest 0.1.1 (Posada [2008](#)) to provide a substitution model for the alignment. Phylogenetic analyses were performed using the Bayesian Inference (BI) and Maximum Likelihood (ML) approaches. Following Vellinga ([2010](#)) and Vellinga et al. ([2010](#)), a *Cystolepiota seminuda* sequence (AY176350) was used as outgroup. The BI was performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist [2001](#)) with four incrementally heated simultaneous Monte Carlo Markov Chains (MCMC) run over 10 million generations, under GTR+ Γ evolutionary model. Trees were sampled every 1,000 generations resulting in an overall sampling of 10,001 trees; the first 2,500 trees were discarded as “burn-in” (25 %). For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). ML estimation was performed through RAxML v.7.0.4 (Stamatakis [2006](#)) with 1,000 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. BI and ML analyses were run on the CIPRES Science Gateway web server (Miller et al. [2010](#)). Only BPP values over 0.70 and MLB over 50 % are reported in the resulting tree (Fig. [1](#)).

Results

Molecular analysis

The amplification of the ITS regions was successful for the four specimens, yielding a PCR product ranging from 669 to 732 bp. The ITS data matrix comprises a total of 63 sequences (including 59 sequences from GenBank). Both analysis methods applied yielded the same topology; therefore, only the Bayesian tree with both BPP and MLB values is shown (Fig. [1](#)). The four sequences of the new *Leucoagaricus* clustered together forming an independent phyletic line not assignable to any other sequenced species of sect. *Piloselli*. The new species emerged in polytomy with the other species of sect. *Piloselli*, in a position not fully resolved.

Taxonomy

Leucoagaricus croceobasis G. Muñoz, A. Caball., Contu & Vizzini, **sp. nov.**

Mycobank MB 807027

Etymology: the specific epithet “*croceobasis*” refers to the saffron tinge (from the Latin *croceus*) of the stipe base.

Original diagnosis:

Macrocharacters (Fig. 2): Pileus 30–70 mm wide, when young hemispherical-convex to hemispherical, expanding to subplane, finally plane, without umbo, with an entire, slightly exceeding margin, involute or incurved in young stages; pileus surface dry, subtomentose and darker at the disc, with variable tinges which could be chestnut-brown, reddish-brown, cream-ochre to cream-rosy (Mu 5YR 4/3–4; 5/4–8), at first completely brown velvety and entire, not squamulose, later diffracted in a velvety-tomentose central calotte with concentric zones of adnate squamules on white or cream background, not or scarcely (and then for a very short time) reddening, without veil remnants. Dried material brown with very light pink or rosy shades. Lamellae free, with a pseudocollarium, crowded ($L = c. 70\text{--}90$), interspersed with lamellulae ($l = (0\text{--})1\text{--}2$), up to 4 mm broad, ventricose, white to cream or slightly beige on age, not reddening when bruised, ochre-cream in dried material, with even or slightly flocculose, concolorous edge. Stipe 40–100 × 5–16 mm, stout, solid, cylindrical, in most specimens with a bulbous base which could be napiform or submarginate and then up to 20 mm wide, more infrequently cylindrical or equal; surface smooth, polished, more rarely slightly fibrillose under the annulus, white, at times dark brownish towards the base typically saffron-tinged (Mu 2.5YR 4/8; 5/8) (Fig. 3e). Annulus simple, membranous, persistent, white, with brown underside. Context fleshy, white, unchanging, typically saffron at the stipe base; smell and taste not peculiar, fungoid, pleasant. Edibility unknown. Spore-print white. Chemical reactions ammonia reaction on pileus, annulus and stipe surface green.



Fig. 2

Leucoagaricus croceobasis. Macroscopic characters. **a–d** Fresh basidiomata in situ. **e** Detail of the saffron-coloured stipe base. **a** from AH-40434 (holotypus); **b** from GM-2609; **c** from GM-2645; **d** from GM-2732; **e** from GM-2645. Scale bars 20 mm

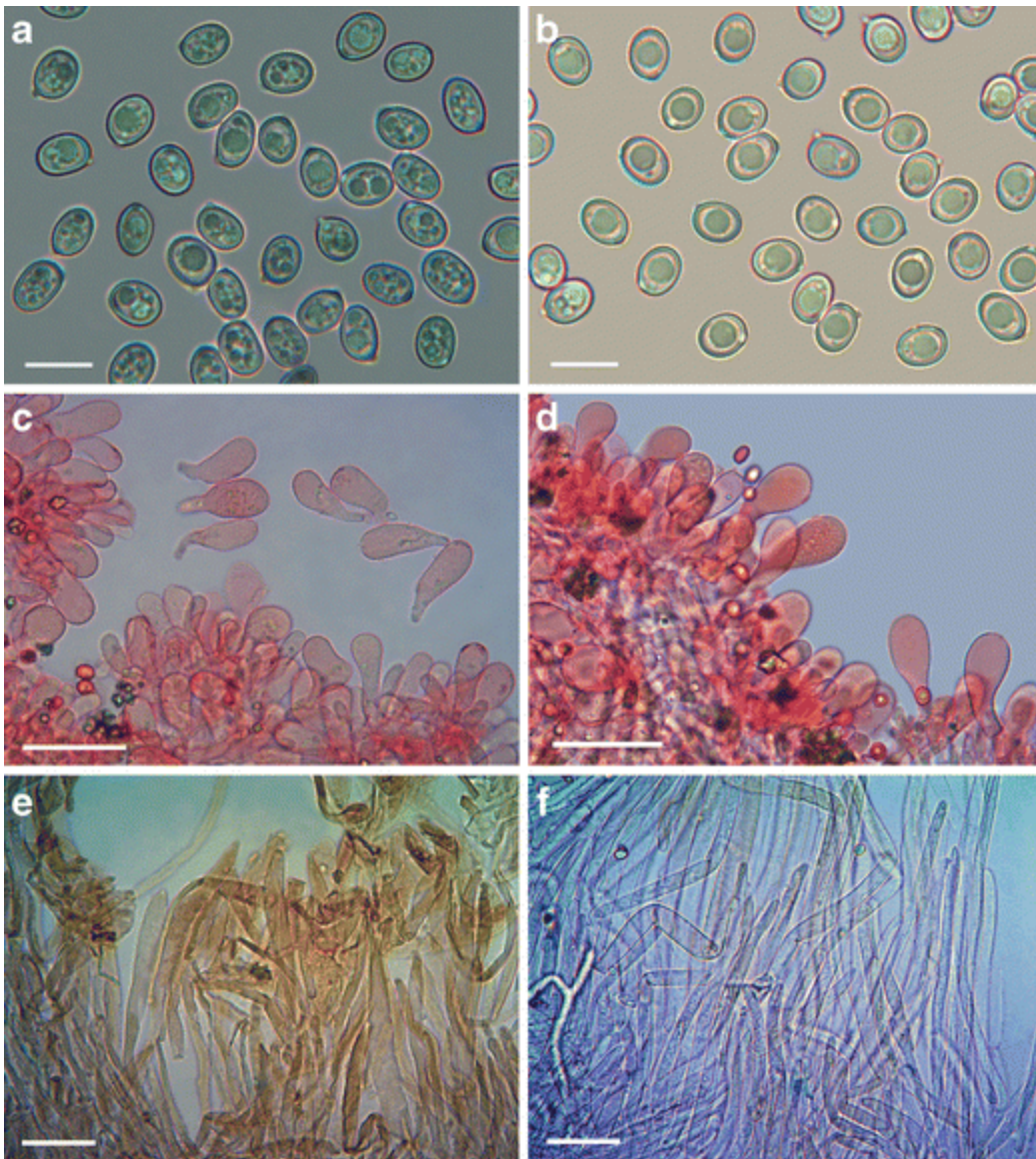


Fig. 3

Leucoagaricus croceobasis. Microscopic characters. **a, b** Spores (in H₂O). **c, d** Cheilocystidia (in ammoniacal Congo red). **e, f** Elements of the pileipellis (in H₂O). **a, c, e** from AH-40434 (holotype); **b, d** from GM-2732; **f** from GM-2609. Scale bars (**a, b**) 10 μm, (**c–f**) 40 μm

Microcharacters (Figs. 3, 4): Spores (6.3–) 6.6–7.4–8.4 (–9.0) × (4.9–) 5.2–5.7–6.1 (–6.4) μm, Q = (1.13–) 1.18–1.31–1.47 (–1.61), n = 153, broadly ellipsoid to ellipsoid, smooth, without a germ pore, dextrinoid, metachromatic in Cresyl Blue (Figs. 3a, b, 4a). Basidia (15–) 18–22 (–25) × (5.5–) 6.0–8.0 (–8.5) μm, four-spored, clavate, without basal clamp connection (Fig. 4b). Lamella edge sterile. Cheilocystidia abundant, (15–) 20–40 (–45) × (7–) 10–15 (–18) μm, clavate, not appendiculate nor mucronate, with the apex typically applanate, hyaline (Figs. 3c, d, 4c). Pleurocystidia absent. Pileus surface a xerotrichoderm consisting of erect, long cylindrical to fusiform elements, never septate, (80–) 100–250 (–350) × (7–) 8–11 (–12) μm, without a subtending (basal) hymeniform layer but with some shorter clavate to subfusiform elements, with a light intracellular (cytoplasmatic), smooth, brownish pigment (Figs. 3e, f, 4d). Clamp-connections absent.

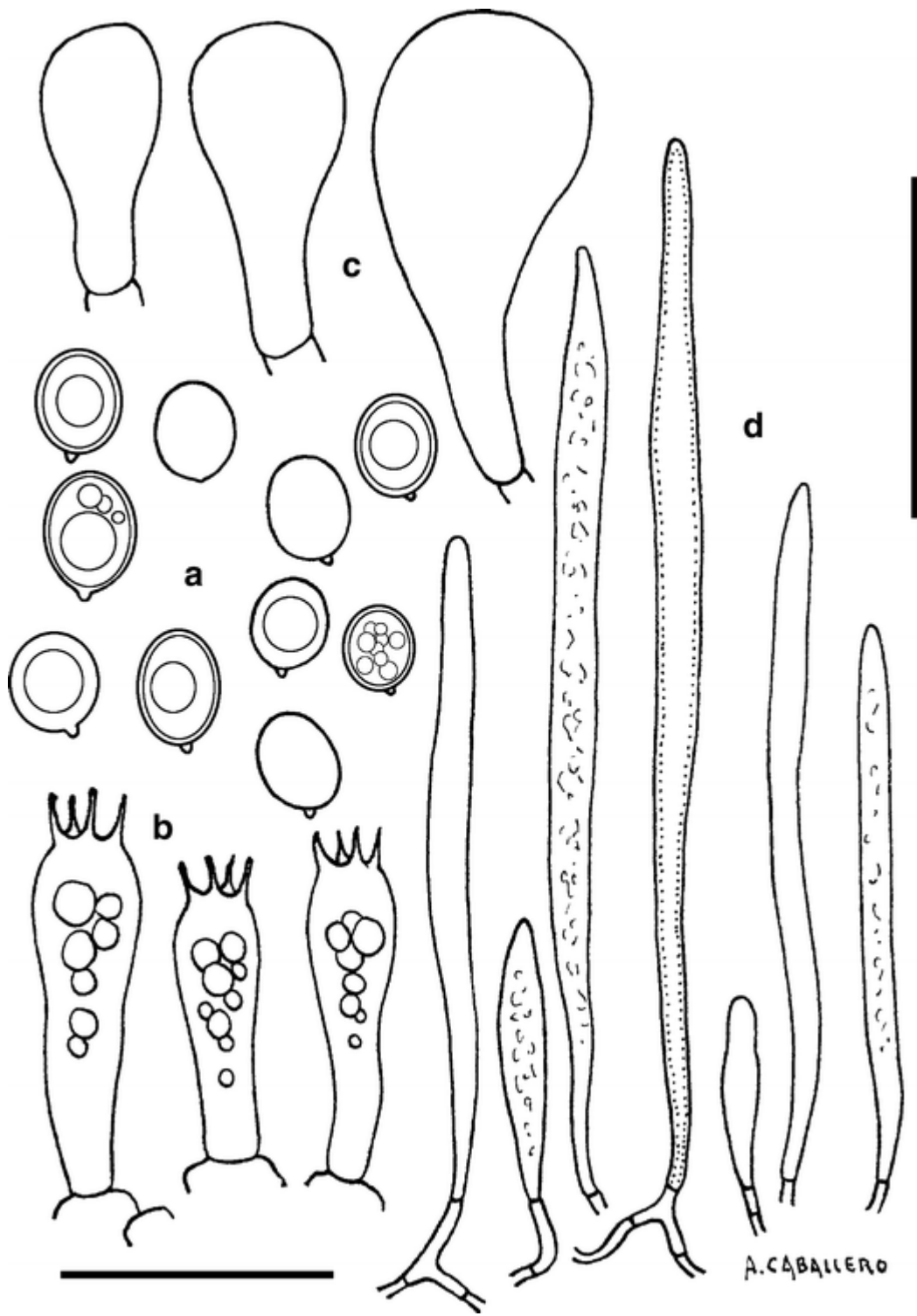


Fig. 4

Leucoagaricus croceobasis. Line drawings of microscopic characters (from AH-40434, holotype). **a** Spores. **b** Basidia. **c** Cheilocystidia. **d** Elements of the pileipellis. Scale bars (**a-c**) 20 μ m, (**d**) 100 μ m

Habitat and distribution — Gregarious, not caespitose, terrestrial, on partially sandy soil, in an public park, at the margin of a small river, in leaf litter among *Populus nigra*, *P. alba*, *Fraxinus excelsior* and *Salix* sp. Fruiting in October-November. Thus far known only from one locality in Spain.

Collections examined: SPAIN, Aragón: Zaragoza, “José Antonio Labordeta” (formerly “Parque Grande”) park, N 41.634 W 0.894, alt. 225 m, 27.XI.2011, G. Muñoz (AH-40434 – **holotype**; isotype in GM-2422); *ibid.*, 9.X.2012, G. Muñoz (GM-2609); *ibid.*, 14.X.2012, G. Muñoz and A. Caballero (GM-2645 and AC-4985); *ibid.*, 14.XI.2012, G. Muñoz (GM-2732).

Discussion

Leucoagaricus croceobasis is easily recognized by having striking distinctive characters: the pileus tinges being chestnut-brown, cream-ochre or reddish-brown in fresh basidiomata, no veil remnants on pileus surface, a saffron-orange stipe base, a context that does not stain orange-red when bruised, broadly ellipsoid to ellipsoid spores, clavate, not appendiculate and not pigmented cheilocystidia and a xerotrichodermic pileipellis consisting of fusiform hyphae with some shorter clavate to subfusiform basal elements.

No other species of *Leucoagaricus* is known in the literature having such a unique combination of macro- and micromorphological features (Candusso and Lanzoni [1990](#); Bon [1993](#); Caballero [1997](#); Gennari and Migliozi [1999](#); Migliozi and Resta [2001](#); Migliozi et al. [2001](#); Vellinga [2001](#), [2006](#), [2010](#)).

Morphologically, *Leucoagaricus variicolor* is the most closely related species: it also possesses broadly ellipsoid spores but sharply differs in having a much paler almost smooth to finely felted-fibrillose pileus, tinged with whitish-pale cream, egg-yellow-ochre to orange-pink in fresh material and pink-rosy in dried specimens and without a saffron-tinged stipe base; additionally, the pileus surface of *L. variicolor* shows abundant, pruinose-fibrillose to submembranose white velar patches as remnants of the universal veil, that are absent in the new species. Micromorphologically, *L. variicolor* shows typically pyriform to sphaeropedunculate (rarely with a very short mucro), often brown-pigmented cheilocystidia, bigger, stouter basidia and intraparietal pigment in the hyphae of the pileus surface (Muñoz et al. [2012](#)). *L. variicolor* was collected in the same public park in Zaragoza as the *L. croceobasis* specimens, but in a different habitat (on argillose-sandy soil, in *Pinus halepensis* litter).

Finally, according to the molecular data, *L. croceobasis* occupies an isolated but not well-resolved position in sect. *Piloselli* (Fig. [1](#)); following the BLASTn search, the most related species (identity value = 89 %) seems to be "*Lepiota*" *fuliginescens* Murrill (see, Vellinga [2010](#) for a recent very detailed study of this species, which is not uncommon in California, USA), but this has a pale grey to pale brown pileus, more elongate spores which are "5.8–8.8 × 3.6–5.2 μm" and larger, appendiculate and pigmented cheilocystidia (Vellinga [2010](#)).

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