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ORIGINAL ARTICLE

Lepiota coloratipes, a new species for *Lepiota rufipes* ss. Auct. europ. non ss. orig.

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Abstract In a recent paper, *Lepiota rufipes* ss. orig. (a North American taxon) was synonymised with *Cystolepiota seminuda*. Accordingly, *Lepiota coloratipes* sp. nov. is here described for the taxon usually referred to as *L. rufipes* in Europe. On the basis of recent collections from Europe and China, a full description, colour pictures of basidiomata, line drawings of microscopic features and ITS phylogenetic analysis are provided. A unique combination of morphological characters—i.e. hymeniform pileus covering, cheilocystidia of variable shape, fugacious partial veil not forming an annulus, presence of oil droplets in all tissues, and uninucleate spores—corresponds to an isolated position of its sequences in the phylogenetic tree.

Keywords *Basidiomycota* · *Agaricales* · *Agaricaceae* · Hymeniform pileus covering · ITS sequences · Taxonomy

Introduction

The structure of the pileus covering has been shown to be a key character in the evolution and infrageneric classification of *Lepiota* (Pers.) Gray (Vellinga and Huijser 1999; Vellinga 2003, 2010). Molecular analysis indicated that the species with a hymeniform pileus covering do not form a monophyletic assemblage (lineage) (Vellinga 2001, 2003, 2010), even though most of them gather in a clade (named clade 3 in Vellinga 2003) which encompasses taxa with different spore shapes (ellipsoid, bullet-shaped to spurred). *Lepiota lilacea* Bres., *L. ochraceofulva* P.D. Orton and *L. pyrochroa* Malençon fall outside this clade and seem to occupy isolated and still unresolved positions (Vellinga 2003), though they share the pileipellis morphology with the other species. This clade corresponds to the morphology-based section *Cristatae* (Kühner ex Wasser) Bon as delimited by Kühner (1936) and Singer (1986) or to sections *Cristatae*, *Integrellae* (Kühner ex Bon) Bon and *Lilaceae* Bon as a whole, distributed over two subgenera by Bon (1993). Bon placed species with a hymeniform pileipellis and bullet-shaped spores in the subgenus *Lepiotula* Maire section *Cristatae*, and he included all the remaining species with the same pileipellis and ellipsoid spores in subgenus *Paralepiotula* Bon. He subdivided the latter subgenus in section *Integrellae* for species with a micaceous, smooth pileus

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surface, which usually does not tear into scales, and have only one nucleus per spore, and section *Lilaceae* for species whose pileus surface breaks into scales and have binucleate spores. Vellinga and Huijser (1999) and Vellinga (2001) included all the species with a hymeniform pileus covering in section *Lilaceae*.

One of the species classified by Vellinga and Huijser (1999) within section *Lilaceae* is *Lepiota rufipes* Morgan, a species described originally from North America (Morgan 1906) with a brief and vague description: "Pileus a little fleshy, convex, smooth and glabrous, white. Stipe slender, smooth and glabrous, rufescent, paler at the summit; the annulus evanescent. Lamellae broad, close, white, free, approximate; spores oblong, 4–5 × 3 mic. Growing on the ground in woods among old leaves; Preston, O. Pileus about a centimeter in diameter, the stipe 2–3 cm. long." Based on Kühner and Maire's (1937) interpretation, most European authors have accepted *L. rufipes* to be a species with a whitish-cream to pale ochraceous or pinkish pileus that does not tear into scales, a reddening stipe base, hymeniform pileus covering, no annulus (but fugacious velar remnants are usually present on stipe surface), small uninucleate spores, and variably shaped cheilocystidia (most narrowly clavate) (Vellinga and Huijser 1999). Smith (1954), in her revision of the Michigan species of *Lepiota*, described *L. rufipes* with features in concordance with those reported by the European authors, with the exception of the pileus "pure white, chalky, becoming sordid in age". *Lepiota rufipes* was not included by Smith (1966) in her studies on North American *Lepiota* type collections.

Finally, Vellinga (2010) was able to locate and to study the type collection of *L. rufipes* (consisting of a single basidiome kept in ISC), and, based on its morphological features (such as an epithelioid pileus covering with globose to subglobose cells, narrow spores and absence of cheilocystidia), *L. rufipes* was placed in synonymy with *Cystolepiota seminuda* (Lasch) Bon. This specimen is to be considered a basidioma of this *Cystolepiota* whose pileus covering sphaerocytes have been washed off by heavy rain.

Consequently, the European "*L. rufipes*", supported by molecular data as a distinct entity among lepiotoid taxa with a hymeniform pileus covering (Vellinga 2003, 2010), is still undescribed.

The aim of the present contribution is to formally describe this species commonly referred to as *L. rufipes*, providing full morphological and molecular data (ITS sequences), based on recent collections from Europe and China.

Materials and methods

Morphology

The description is mainly based on the examination of two collections from Spain (15 basidiomata in all development stages) found at two different sites and supported by supplementary material from Italy, France and China. The data on voucher specimens are presented in their original form.

The macromorphological characters were observed on fresh material, the micromorphological characters in dried material under an Olympus CX41 light microscope with an oil immersion lens. Sections were rehydrated in 5% KOH, and then separately mounted in ammoniacal Congo Red, Cotton Blue and Melzer's reagent. Spores were also mounted in Cresyl Blue to test for a metachromatic reaction (Singer 1986). The fluorochrome 4',6-diamidino-2-phenyl-indoldihydrochloride (DAPI) was used to stain nuclei in spores following Horton (2006). The number of nuclei in spores was then determined using a Nikon E800 epifluorescence microscope.

For the measurements of microcharacters (spores, cheilocystidia, basidia and terminal elements of pileipellis) minimum, maximum (in parentheses) and average ± standard deviation values are presented. Spore size refers to mature (thick-walled) spores only. The following abbreviations are used: L = number of lamellae reaching the stipe; l = number of lamellulae between each pair of lamellae; [X, Y, Z] indicating that measurements were made on X spores, in Y samples from Z collections; Q = the spore quotient (length/width ratio); Qav = the average spore quotient. Terminology for descriptive terms is according to Vellinga (2001). Colour designations are from Kornerup and Wanscher (1974). Herbarium abbreviations follow Thiers (2013, continuously updated) except "LP" that refers to the personal herbarium of Luigi Perrone (Rome). The holotype collection is kept in SAV. Author citations follow the Index Fungorum – Authors of Fungal Names (http://www.indexfungorum.org/authorsoffungalnames.htm).

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was isolated from 1 mg of four herbarium specimens (SAV F-3212, SAV F-3213, HKAS 52268 and HKAS 52305) using the DNeasy Plant Mini Kit (Qiagen, Milan, Italy). Universal primers ITS1F/ITS4 were used for the ITS region amplification (White et al. 1990; Gardes and Bruns 1993). Amplification reactions were performed in PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in a 25-μl reaction mixture using the following final concentrations or total amounts: 5 ng DNA, 1× PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 μM of each primer, 2.5 mM MgCl2, 0.25 mM of each dNTP, 0.5 unit of Taq polymerase (Promega). The PCR program was as follows: 3 min at 95 °C for 1 cycle; 30 s at 94 °C, 45 s at 50 °C, 2 min at 72 °C for 35 cycles, and 10 min at 72 °C for 1 cycle. PCR products were resolved on a 1.0 % agarose gel and visualised by staining with ethidium bromide. PCR products were purified and sequenced by MACROGEN (Seoul, Republic of Korea) and

Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). Sequence assembly and editing were performed using Geneious v.5.3 (Drummond et al. 2010). The sequences are deposited in GenBank under the accession numbers given in Fig. 1.

Sequence alignment and phylogenetic analysis

The sequences obtained in this study were compared to those available in the GenBank database (http://www.ncbi.nlm.nih.gov/) by using the Blastn algorithm. Based on the Blastn results, sequences were selected according to the outcomes of recent phylogenetic studies on *Lepiota* (Vellinga 2003, 2010). Besides *Lepiota* species with a hymeniform pileus covering, eight species (indicated by an asterisk in Fig. 1) representative of the major clades in *Lepiota* as delimited by Vellinga (2003) were chosen for comparison.

Alignments were generated using MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The sequence alignments were then imported into MEGA 5.10 (Tamura et al. 2011) for manual adjustment. *Chamaemyces fracidus* (Fr.) Donk (AY176343) was used as outgroup taxon because it is basal in the *Agaricaceae* (Vellinga 2004, 2010). 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)

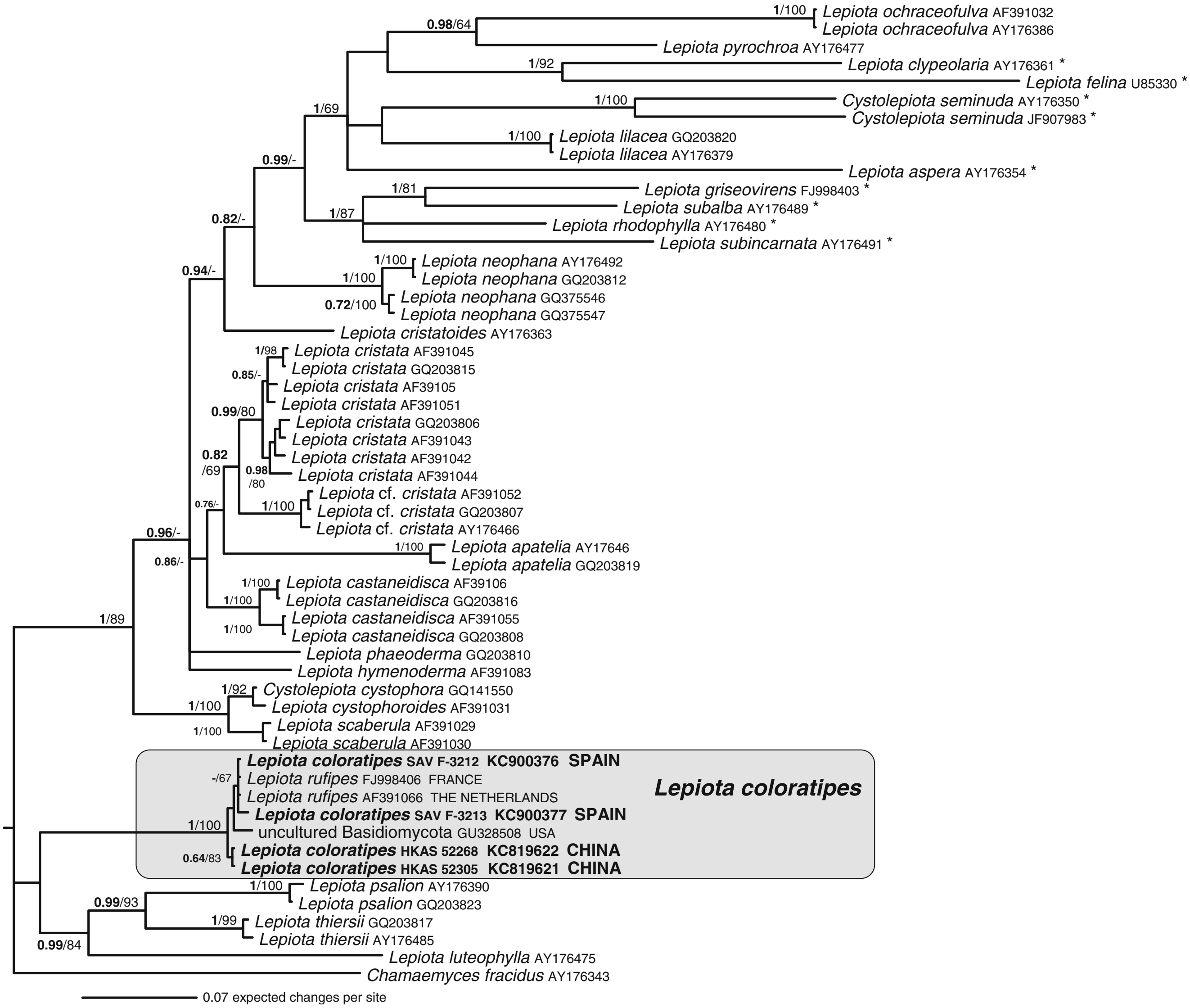

Fig. 1 Bayesian phylogram obtained from the general nrITS sequence alignment of *Lepiota* spp. Included are: *Lepiota* species with a hymeniform pileus covering, eight species representative of the major clades in *Lepiota* (indicated by *) and *Chamaemyces fracidus* as outgroup taxon. Support values in either the Bayesian [Posterior Probabilities values (BPP)] or Maximum Likelihood [ML Bootstrap percentage (MLB)] analyses are indicated. Only BPP values over 0.70 (in *bold*) and MLB values over 50 are given above clade branches. Newly sequenced collections are in *bold*

and Maximum Likelihood (ML) approaches. 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 the 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.7.3.2 (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). Branch lengths were estimated as mean values over the sampled trees.

Pairwise % identity values of ITS sequences were calculated using MEGA 5.10 (Tamura et al. 2011).

Fig. 2 *Lepiota coloratipes*. Basidiomata. **a** Holotype, SAV F-3213, photo by Per Marstad; **b** SAV F-3212, photo by Soňa Jančovičová; **c** LP, photo by Luigi Perrone. *Scale bars* 10 mm

Results

Molecular analysis

Both Bayesian and Maximum Likelihood analyses produced the same topology; therefore, only the Bayesian tree with both BPP and MLB values is shown (Fig. 1). The ITS data matrix comprised a total of 55 sequences (including 51 from GenBank). The alignment comprised 816 characters, and contains 397 variable sites.

In the obtained Bayesian phylogram (Fig. 1), our four *Lepiota coloratipes* sequences (two from Spain and two from China) clustered with two sequences named "*Lepiota rufipes*" (AF391066, collection from The Netherlands, herb. Huijser; FJ998406, collection from France, MCVE 16888, originally labelled as *Lepiota parvannulata*) and one sequence from an environmental sample (GU328508, uncultured Basidiomycota clone man22_soil_G02, from soil, Michigan, USA), forming a well-supported clade (BPP = 1 and MLB = 100) with a pairwise % identity value of 99.4.

Taxonomy

Lepiota coloratipes Vizzini, J.F. Liang, Jančovičová & Zhu L. Yang, sp. nov. (Figs. 2, 3, 4, 5 and 6)

MYCOBANK MB 804096

= *Lepiota rufipes* f. *phaeophylla* Bon in Bon and Riousset, Doc. Mycol. 22(85): 69 (1992)

– *Lepiota rufipes* Auct. non Morgan: Kühner and Maire, Bull. Soc. Hist. Nat. Afrique N. 28: 108–109 (1937); Josserand, Bull. Soc. Mycol. France 71: 65–67 (1955); Einhellinger, Ber. Bayer. Bot. Ges. 41: 98 (1969); Lanzoni, Boll. Gruppo Micol. Bres. 29: 81–83 (1986); Candusso and Lanzoni, Fungi Europaei 4: 320–322 (1990); Kelderman, Coolia 35: 76–79 (1992); Bizio et al., Riv. Micol. 36: 239–240 (1993); Bon, Flore Mycologique d'Europe 3: 82 (1993); Kelderman, Parasolzwammen van Zuid-Limburg Nederland: 3.2 $^{9.4}$ (1994); Vellinga and Huijser, Belg. J. Bot. 131: 204–205 (1999); Vellinga, Flora Agaricina Neerlandica 5: 143–144 (2001); Mohr, Boletus 30: 76 (2008); Lange in Knudsen and Vesterholt, Funga Nordica Ed. 2: 540 (2012); Ludwig, Pilzkompendium 3, Beschreibungen: 426–427 (2012)

EXCLUDED:

Lepiota rufipes Morgan, J. Mycol. 12: 156 (1906) [= *Cystolepiota seminuda* (Lasch) Bon]

Lepiota rufipes Auct. non Morgan: Babos, Ann. Hist.-Nat. Mus. Natl. Hung. 66: 65–67 (1974); Wasser, Flora Gribov Ukrainy. Agarikoyve Griby (Kiev): 255–257 (1980); Krieglsteiner, Beitr. Kenntn. Pilze Mitteleur. 7: 69–71 (1991) [= *L. psalion* Huijser & Vellinga]

Lepiota rufipes f. *phaeophylla* Auct. non Bon: Bizio et al., Riv. Micol. 36: 240–242 (1993) [= *L. cristatoides* Einhell.]

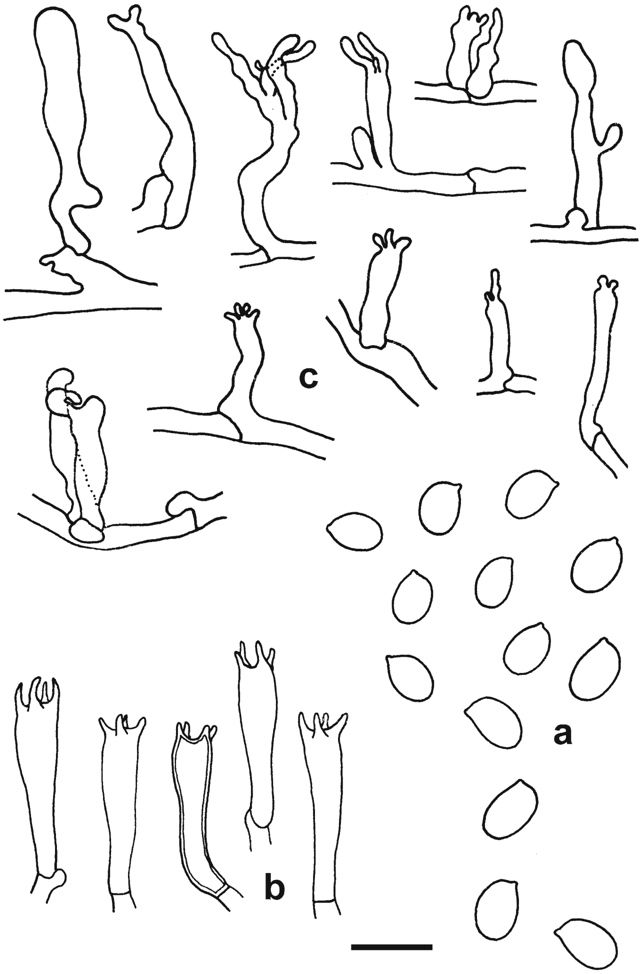

Fig. 3 *Lepiota coloratipes*. Microscopic characters (from holotype). **a** Spores; **b** basidia; **c** caulocystidia. Line drawings by Slavomír Adamčík. *Scale bars* (**a**) 5 μm, (**b**, **c**) 10 μm

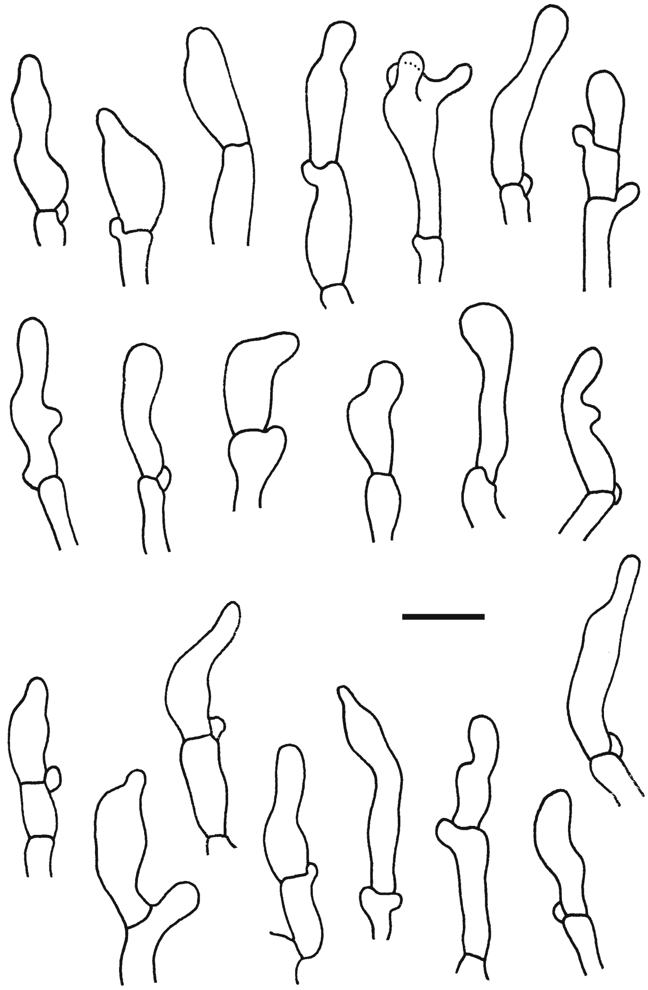

Fig. 4 *Lepiota coloratipes*. Microscopic characters (from holotype). Cheilocystidia. Line drawings by Slavomír Adamčík. *Scale bar* 10 μm

Lepiota “cf. *rufipes* f. *phaeophylla*“: Winterhoff & Bon, Carolinea 52: 8 (1994) [= *L. psalion* Huijser & Vellinga]

SEL. ICON.: Moser and Jülich, Farbatlas der Basidiomyceten, III *Lepiota*: 9 (1985); Lanzoni, Boll. Gruppo Micol. Bres. 29: p. 84 (1986); Candusso & Lanzoni, *Lepiota*, Fungi Europaei 4: Pl. 35b (1990); Bizio et al., Riv. Micol 36: p. 239 (1993); Mohr, Boletus 30: Pl. 17 (2008); Eyssartier and Roux, Le guide des champignons France et Europe: p. 316 (2011); Ludwig, Pilzkompendium. Band 3. Abbildungen: Tab. 551 n. 111.13 (2012) [All as *L. rufipes*].

Etymology the specific epithet refers to the coloured (pale brown-reddish) stipe.

Original diagnosis:

Pileus usque ad 30 mm latus, e convexo planus ac subumbonatus; nec hygrophanus nec margine striatus; pileus fere totus albus, mox discum versus leviter brunneo maculatus, in adultis speciminibus brunneolus, margine eburneus, vel saepe ambitu viridi variegatus; superficies sicca, laevis in juvenilibus speciminibus, dein concentice zonatimque rimosa. Stipes 15–27(40) × 1.5–2.5(−3) mm centralis, cylindraceus, vulgo aequalis, ab initio albus, posterius aurantio-usque brunneo-cinereus distincte vel indistincte subincarnato colore tinctus, basi obscurior aurantio-brunneus, sursum deorsum subtiliter sericeo-fibrillosus, primo aetate cavus, externe tenuibus fibrillis cortinalibus praeditus. Lamellae subliberae vel leviter adnatae brunneo-griseae vel eburneolae, acies concolor. Caro pilei alba, stipitis cum ejus superficie concolor, lenta odore saporeque proprio destituta. Sporae (3−)3.2–3.6(−4) × (2−)2.3–2.7(−2.8) μm, plus minusve 3.4 × 2.5 μm, ellipsoideae, hyalinae, laeves, tenuiter tunicatae, metachromaticae, haud amyloideae nec dextrinoideae, nec cyanophilae. Basidia tetraspora 18.5–22 × 4.5–5.5 μm. Cheilocystidia (10.5−)15–25(−29) × (4.5−)5–7(−8) μm, versiformia plerumque lageniformia vel lecythiformia, hyalina tenuiter tunicata. Cutis pilei hymeniformis ex uno strato cellularum terminalium inflatarum constituta, ope KOH (5%) prope marginem distincte flavoviridis; in junioribus basidiomatibus terminales cellulae (15−)26.5–42.5(−54) × (5.5−)9–14.5(−18) μm, plus minusve 34.3 × 11.7 μm, in adultioribus paulo majores

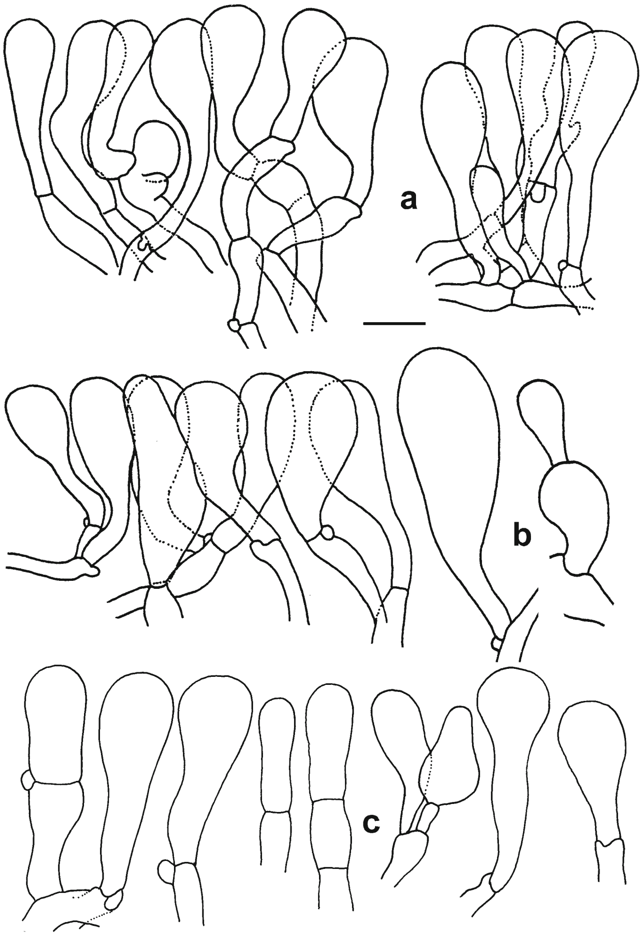

Fig. 5 *Lepiota coloratipes*. Microscopic characters. Pileus covering elements (from holotype). **a**, **b** Terminal elements in the pileus margin; **c** terminal elements in the pileus centre. Line drawings by Slavomír Adamčík. *Scale bar* 10 μm

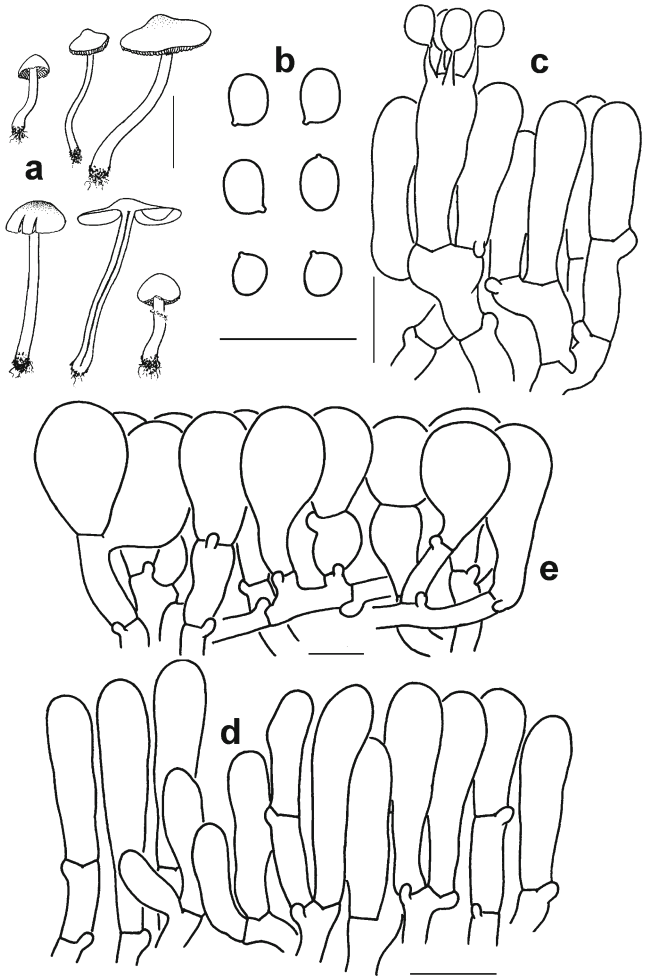

Fig. 6 *Lepiota coloratipes*. Habitus and microscopic characters (from HKAS 52305). **a** Basidiomata; **b** spores; **c** basidia; **d** cheilocystidia; **e** pileus covering. Line drawings by Zhu L. Yang. *Scale bars* (**a**) 20 mm; (**b**–**e**) 10 μm

sphaero- vel clavato-pedunculatae; pileipellis e minoribus terminalibus cellulis constituta, ope KOH fortius decolorans in medio pilei. Suprapellis deest, quoad subpellem circa 20μm crassa est figuraque pseudoparenchymatis. Caulocystidia solum ad apicem stipitis adsunt, saepe aborta basidia in mentem revocantes. Omnibus in partibus ubique hyphae fibulatae.

Holotypus (hic designatus): SAV F-3213.

Detailed description - Macrocharacters PILEUS up to 30 mm wide, at first campanulate, hemispherical or broadly conical, later plano-convex to applanate, subumbonate; not hygrophanous; margin not striated when wet and dry, when young with overhanging cuticle in the form of irregular teeth on the margin; variable in colour, the pileus is almost completely white when young, soon becomes brownish spotted in the centre, when mature turns light brown (5D4, 5D5), camel (6D4), brown (6E4) to dark brown (6F6) in the centre, brownish orange (5C3) towards margin, but always paler ivory (4B3) to white at the margin; often with green spots (2D4, 1E4, 1E3) near margin or sometimes emarginated with narrow green line; surface dry, when young usually smooth, micaceous, later cracking in concentric zones and revealing white context as it ages or dries, when old sometimes squarrulose. STIPE 15–27(40) × 1.5–2.5(−3) mm, central, cylindrical, usually regular, but occasionally also slightly flexuous, hollow; when young white, later orange gray (6B2) to brownish gray (6C2), with indistinct or distinct pink shade, on the base darker brown orange (6C3); finely silky fibrillose along all length; stipe and pileus margin connected initially with fine whitish fibrils, forming an indistinct ring zone on the upper part of the stipe that soon disappears; at the base sometimes with white tomentum and white ramified minute rhizomorphs. LAMELLAE 2–3 mm wide, L = 32–44, l = 1–3, subfree to adnexed, brownish gray (6C2) or ivory (4B3), without any violaceous or pinkish tints, edge finely granulose (under lens), almost white. CONTEXT in pileus white; in stipe concolorous with its surface, but white in central cavity; elastic; without specific smell and taste. SPORE PRINT pale cream.

Microcharacters SPORES [60, 2, 2] (3−)3.2–3.6(−4) × (2−)2.3–2.7(−2.8) μm, on average 3.4 × 2.5 μm, Q = (1.25−)1.3–1.45(−1.5), Qav = 1.38, ellipsoid, hyaline, thin-walled, smooth but sometimes verruculose in Melzer's reagent, when young and when mature metachromatic in Cresyl Blue, non-amyloid, non-dextrinoid, non cyanophilic in Cotton Blue, uninucleate. BASIDIA 4-spored, 18.5–22 × 4.5–5.5 μm, hyaline, thin-walled; thick-walled basidia absent or very sparse, usually occur close to the lamellar edge, indistinctly dextrinoid. CHEILOCYSTIDIA (10.5−)15–25(−29) × (4.5−)5–7(−8) μm, hyaline, thin-walled, various in shape in all parts of the lamellae edge, mostly lageniform or lecythiform, occasionally fusiform or clavate, occasionally with multiple lateral projections or nodules, sometimes uniseptate and moniliform. PLEUROCYSTIDIA absent. PILEUS COVERING (PILEIPELLIS) a hymeniderm, composed of one layer of inflated, tightly packed terminal elements, near the margin of pileus, distinctly green-yellow in 5% KOH, terminal cells in younger basidiomata (15−)26.5–42.5(−54) × (5.5−)9–14.5(−18) μm, av. 34.3 × 11.7 μm, in older basidiomata somewhat larger, av. 38.1 × 13.6 μm, sphaeropedunculate or clavate-pedunculate; pileipellis in the centre of pileus with more distinct KOH reaction, terminal elements smaller, av. 24.2 × 10 μm, thick-walled (cell walls ca. 0.5 μm), with walls embedded in gelatinous matter, which makes it difficult to trace the basal septa and lower structure in the microscope; subpellis ca. 20 μm thick, pseudoparenchymatic, composed of densely arranged twisted hyphae that are 3–7 μm thick. HYMENOPHORAL TRAMA subregular, comsisting of hyphae 4–15 μm wide, mostly wider than 10 μm; trama of pileus and stipe similar to that in lamellae. CAULOCYSTIDIA present only at apex of stipe, absent towards the base, 3.5–5 μm thick, cylindrical, moniliform, with an apical projection and often reminiscent of aborted basidia, hyaline, thin-walled. CLAMP CONNECTIONS present in all tissues. EXTRACELLULAR OIL DROPLETS abundant in all tissues and on spore surface.

Habitat and distribution in small groups, terrestrial, on loamy or sandy humus-rich soils, in deciduous or coniferous woods, urban parks (and then often under *Cedrus* spp.). Summer to winter. Rare but widespread in Europe, also in East Asia (Yunnan prov., China) and North America (see Discussion).

Examined material SPAIN, Mas dels Pilars, Ares del Maestre municipality, province of Castellón, calcareous clay soil, pH 7.7, quaternary limestone, among fallen leaves under *Quercus ilex*, associated with *Thymus vulgaris*, *Calicotome spinosa*, *Carex halleriana*? and *Euphorbia characias*, UTM S30 X 741542 Y 4485041 Z 1015, 21 Oct 2010, leg. S. Adamčík & S. Jančovičová (SAV F-3212; GenBank acc. n. KC900376); SPAIN, Morella region, Ares del Maestre municipality, Pinar del Hostal de la Rocha, on calcareaous argilaceous soil, along forest road, associated with *Pinus nigra* subsp. *nigra*, *P. nigra* subsp. *salzmannii* and *Brachypodium phoenicoides*, UTM S30 X 743085 Y 4487390 Z 973, 22 Oct 2010, leg. F. Paci (SAV F-3213, HOLOTYPE; GenBank acc. n. KC900377).

Additional examined material ITALY, Pineta San Nicolò, IGM 128-3-VE, 27 Nov 1997, leg. E. Bizio, det. G. Zecchin (MCVE 13323); Vaiont PN, IGM 065.NO, 250 m a.s.l., 26 Sept 2010, on *Cedrus* sp. litter, leg. & det. G. Zecchin" (MCVE 24846); Vaiont, PN, IGM 065.NO, 250 m a.s.l., on *Cedrus* sp. litter, leg. & det. G. Zecchin (MCVE 26891); Parco pubblico del Comprensorio "Roma 70", quart. Ardeatino, Rome, on *Laurus nobilis* litter, 21 Nov 2012, leg. L. Perrone (LP). FRANCE, Martigues, Marseille, 12 Nov 1999, leg. A. Gennari & G. Robich, det. A. Gennari (as *L. parvannulata*, MCVE16888, GenBank acc. n. FJ998406). CHINA, Yunnan Prov., Kunming, Kunming Institute of Botany, Chinese Academy of Sciences, 1,900 m a.s.l., 1 Aug 2007, leg. Zhu L. Yang 4790 (HKAS 52305; GenBank acc. n. KC819621); 7 Sept 2007, leg. Zhu L. Yang 4951 (HKAS 52268; GenBank acc. n. KC819622); 18 Aug 2008, leg. Zhu L. Yang 5163 (HKAS 54460); 20 Oct 2010, leg. Zhu L. Yang 5510 (HKAS 59238); 8 Aug 2012, leg. Zhu L. Yang 5622 (HKAS 75443).

Discussion

Distinguishing features

Lepiota coloratipes is clearly distinguished from the other taxa with a hymeniform pileus covering by having a peculiar combination of characters including: a usually smooth pileus surface with cream to pale ochraceous tinges, a fugacious partial veil not forming an annulus but leaving fibrillose remnants on stipe surface, a stipe with brownish-reddish tinges at base, the presence of oil droplets in all tissues (including spore surface), the hymeniform pileus covering consisting of a tightly arranged palisade of more or less clavate to sphaeropedunculate elements, the presence of uninucleate spores (versus the binucleate status of all the other *Lepiota* species with a hymeniform pileus covering), variably shaped cheilocystidia, and a unique ITS sequence. Our description based on collections from Europe and China, fits well with those from most European authors (e.g. Kühner and Maire 1937; Kühner 1945; Josserand 1955; Einhellinger 1969; Lanzoni 1986; Candusso and Lanzoni 1990; Kelderman 1992, 1994; Bizio et al. 1993; Bon 1993; Vellinga and Huijser 1999; Vellinga 2001; Mohr 2008; Lange 2012; Ludwig 2012a, b). The spores of *L. coloratipes* could appear slightly verrucose when observed in Melzer's reagent (e.g. Kelderman 1992, 1994; Bon 1993; Vellinga and Huijser 1999; Vellinga 2001), but according to Vellinga and Huijser (1999) they are smooth when analysed with scanning electron microscopy. This rough aspect could be due to the presence of small oil droplets on the spore surface.

This species is quite rare but widespread in Europe, and it is known from several localities in Kunming Institute of Botany in China. Smith's description (1954) of *Lepiota rufipes* from Michigan (USA) corresponds well with our species described here. We have confirmed its occurrence in North America, since the environmental ITS sequence from Michigan soil (uncultured Basidiomycota, GU328508) clusters with the European and Chinese sequences of *L. coloratipes* in the molecular analysis (Fig. 1).

Lepiota rufipes f. *phaeophylla* Bon was established for a taxon sharing nearly all the distinctive features of the type (uninucleate spore included), the exception being the darker, ochre-brown lamellae and variable cheilocystidia; the presence of an annulus is not reported (Bon and Riousset 1992). Vellinga and Huijser (1999) who studied the holotype (herb. Bon 771029) found variably shaped cheilocystidia, sometimes moniliform, a feature typical of *L. coloratipes* as well. This taxon is here considered only a colour form of "*L. rufipes*" without taxonomic value (not deserving taxonomic recognition). *Lepiota rufipes* f. *phaeophylla* sensu Bizio et al. (1993) corresponds to *L. cristatoides* according to Vellinga and Huijser (1999) and Vellinga (2001). *Lepiota* "cf. *rufipes* f. *phaeophylla*" sensu Winterhoff and Bon (1994) and *L. rufipes* sensu Babos (1974), Wasser (1980) and Krieglsteiner (1991), all with a distinct annulus, are referable to *L. psalion* (Vellinga and Huijser 1999; Vellinga 2001).

Allied species

As already stated by Kühner and Maire (1937), Smith (1954) and Josserand (1955), *L. coloratipes* in the field might be confused with *Cystolepiota seminuda*; the latter species differs by an epithelioid pileus covering (abundant pluristratous sphaerocytes), narrower spores and absence of cheilocystidia (Candusso and Lanzoni 1990; Bon 1993; Vellinga 2001). Among the other morphologically similar species, *L. psalion* Huijser & Vellinga is characterised by a distinct well-formed annulus, a looser pileipellis structure, absence of oil-like droplets in tissues and binucleate spores (Vellinga and Huijser 1999; Vellinga 2001). *Lepiota cristatoides* differs in a squamulose pileus surface, the presence of an annulus, the absence of cheilocystidia, more elongated spores, and the binucleate spores (Einhellinger 1973; Vellinga and Huijser 1999; Hausknecht and Pidlich-Aigener 2005); *L. neophana* var. *europaea* Bizio & Migl. and f. *papillata* Migl. & L. Perrone have longer binucleate spores, few clamp-connections in pileus trama and no cheilocystidia (Anonymous 1992; Bizio et al. 1993; Vellinga and Huijser 1999). *Lepiota apatelia* Vellinga & Huijser and *L. thiersii* Sundb. (the latter from western North America) are distinguished by a diffracted pileus covering, a small annulus, ellipsoid binucleate spores, and the absence of cheilocystidia (Sundberg 1989; Vellinga and Huijser 1999; Hausknecht and Pidlich-Aigener 2005; Kosakyan et al. 2008; Gierczyk et al. 2011).

Finally, *L. pyrochroa* Malenç. shares with *L. coloratipes* the micaceous pileus surface due to the tightly packed claviform elements of pileipellis, oil droplets in tissues, and rough spores when studied in Melzer's reagent, but differs in having an orange-red pileus and stipe, and binucleate spores (Malençon and Bertault 1970; Riousset and Josserand 1976; Bon and Riousset 1992; Bizio et al. 1993; Antonín and Vágner 1998; Vellinga and Huijser 1999). Our phylogenetic analysis (Fig. 1) has pointed out, as already stated by Vellinga (2003, 2010), that these two species are not closely related.

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References

Anonymous (1992) Nova taxa in *Lepiota* s.l. Boll Ass Micol Ecol Romana 27:44–45

Antonín V, Vágner A (1998) [1997] New, rare and less known macromycetes in Moravia (Czech Republic) – IV. Acta Mus Moraviae Sci Biol 82:29–38

Babos M (1974) Studies on Hungarian *Lepiota* s.l. species, IV. Ann Hist-Nat Mus Natl Hung 66:65–75

Bizio E, Migliozzi V, Zecchin G (1993) La sezione *Integrellae* (Kühner ex M. Bon) M. Bon del genere *Lepiota* (Persoon) Gray. Riv Micol 36:223–244

Bon M (1993) Flore mycologique d'Europe, 3. Les Lépiotes. *Lepiotaceae* Roze. Doc Mycol Mémoire hors série no. 3. L'Association d'Ecologie et Mycologie, Lille

Bon M, Riousset G (1992) Lepiotes méridionales ou thermophiles, nouvelles ou intéressantes (1 – Tribu *Lepiotae*, genre *Lepiota*). Doc Mycol 22(85):63–73

Candusso M, Lanzoni G (1990) *Lepiota* s.l. Fungi Europaei 4. G. Biella, Saronno

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2010) Geneious v5.3. Available from http://www.geneious.com/

Einhellinger A (1969) Die Pilze der Garchinger Heide. Ber Bayer Bot Ges 41:79–130

Einhellinger A (1973) Die Pilze der Pflanzengesellschaften des Auwaldgebietes der Isar zwischen München und Grüneck. Ber Bayer Bot Ges 44:5–99

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Gierczyk B, Kujawa A, Szczepkowski A, Chachuła P (2011) Rare species of *Lepiota* and related genera. Acta Mycol 46:137–178

Hausknecht A, Pidlich-Aigener H (2005) *Lepiotaceae* (Schirmlinge) in Österreich 2. Die Gattung *Lepiota*. Österr Z Pilzk 14:41–78

Horton TR (2006) The number of nuclei in basidiospores of 63 species of ectomycorrhizal *Homobasidiomycetes*. Mycologia 98:233–238

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755

Josserand M (1955) Notes critiques sur quelques champignons de la région Lyonnaise, 5. Bull Soc Mycol Fr 71:65–125

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl Acids Res 30:3059–3066

Kelderman PH (1992) Nieuwe en zeer zeldzame Nederlandse Lepiota-soorten (7). Coolia 35:76–82

Kelderman PH (1994) Parasolzwammen van Zuid-Limburg Nederland. *Lepiota* s.l. excl. *Macrolepiota*. Stichting Natuurpublicaties, Limburg

Kornerup A, Wanscher JH (1974) Farver i Farver. Politikens, København

Kosakyan A, Ur Y, Wasser SP, Nevo E (2008) Rare and noteworthy lepiotaceous species (*Basidiomycota*, *Agaricales*, *Agaricaceae*) from Israel. Mycotaxon 103:59–74

Krieglsteiner GJ (1991) Über neue, seltene, kritische Makromyzeten in Westdeutschland (ehemalige BR Deutschland, Mitteleuropa). XII. Röhrlinge und Blätterpilze. Beitr Kenntnis Pilze Mitteleur 7: 61–79

Kühner R (1936) Recherches sur le genre *Lepiota*. Bull Soc Mycol Fr 52:177–238

Kühner R (1945) Nouvelles recherches sur les divisions nucléaires dans la baside et les spores des Agaricales. CR Acad Sci Paris 220:618–620

Kühner R, Maire R (1937) Trois Lépiotes peu connues. Bull Soc Hist nat Afr N 28:108–112

Lange C (2012) *Lepiota* (Pers. : Fr.) Gray. In: Knudsen H, Vesterholt J (eds), Funga Nordica. Agaricoid, boletoid, clavarioid, cyphelloid and gastroid genera. Nordsvamp, Copenhagen, pp 627–637

Lanzoni G (1986) Raccolte interessanti del 1984. Boll Gruppo Micol Bres 29:81–90

Ludwig E (2012a) Pilzkompendium. Band 3. Beschreibungen. Fungicon, Berlin

Ludwig E (2012b) Pilzkompendium. Band 3. Abbildungen. Fungicon, Berlin

Malençon G, Bertault R (1970) Flore des champignons supérieurs du Maroc, Tome I. Faculté des Sciences de Rabat, Rabat

Miller MA, Pfeiffer W, Schwartz T (2010) "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, pp 1–8

Mohr P (2008) Interessante Schirmlinge aus Berlin und der Mark Brandenburg. Boletus 30(2):47–79

Morgan AP (1906) North American species of *Lepiota*. J Mycol 12:154–159, 195–203; 242–248

Posada D (2008) jModeltest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Riousset G, Josserand M (1976) Récolte en France d'un basidiomycète marocain. "*Lepiota pyrochroa* Mal. Bull Mens Soc Linn Lyon 45:198–200

Singer R (1986) The *Agaricales* in modern taxonomy, 4th edn. Koeltz, Koenigstein

Smith HV (1954) A revision of the Michigan species of *Lepiota*. Lloydia 17:307–328

Smith HV (1966) Contributions toward a monograph on the genus *Lepiota* I. Type studies in the genus *Lepiota*. Mycopathol Mycol Appl 29:97–117

Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690

Sundberg WJ (1989) *Lepiota* sensu lato in California. III. Species with a hymeniform pileipellis. Mycotaxon 34:239–248

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Thiers B (2013) [continuously updated]. Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

Vellinga EC (2001) *Lepiota* (Pers. : Fr.) S.F. Gray. In: Noordeloos ME, Kuyper TW, Vellinga EC (eds) Flora Agaricina Neerlandica. Critical monographs on families of agarics and boleti occurring in the Netherlands 5. Balkema, Lisse, pp 109–151

Vellinga EC (2003) Phylogeny of *Lepiota* (*Agaricaceae*)—evidence from nrITS and nrLSU sequences. Mycol Prog 2:305–322

Vellinga EC (2004) Genera in the family *Agaricaceae*—evidence from nrITS and nrLSU sequences. Mycol Res 108:354–377

Vellinga EC (2010) *Lepiota* in California: species with a hymeniform pileus covering. Mycologia 102:664–674

Vellinga EC, Huijser HA (1999) [1998] Studies in *Lepiota* I. Species with a hymeniform pileus covering. Belg J Bot 131:191–210

Wasser SP. 1980. Flora gribov Ukrainy, Agarikovye griby. [Fungal Flora of the Ukraine: Agaricoid Fungi]. Naukova Dumka, Kiev

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand D, Sninsky J, White T (eds) PCR Protocols, a guide to methods and applications. Academic, Orlando, pp 315–322

Winterhoff W, Bon M (1994) Zum Vorkommen seltener Schirmlinge (*Lepiota* s.l.) im nördlichen Oberrheingebiet. Carolinea 52:5–10