

Two new species and a new record of *Lepiota* (*Basidiomycota*, *Agaricales*) from the Dominican Republic

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Abstract Two new species of *Lepiota* (*L. squamulodiffracta* and *L. sosuensis*) and a new record (*L. subgranulosa*) are described based on collections made in the Dominican Republic. The phylogenetic position of the species based on nrITS data is analyzed and discussed. *L. squamulodiffracta* belongs in section *Lepiota*, and it is characterized by the velar patches on pileus split up into numerous minute squamules, striate-sulcate pileus up to the center, and penguin-shaped spores. *L. sosuensis* has overall white basidiocarps, dextrinoid and metachromatic spores, trichodermial pileus covering, and rare clamp-connections. *L. subgranulosa* stands out morphologically by the spurred spores with one or several protuberances that give the spores an irregular outline.

Keywords *Agaricaceae* · Neotropics · nrITS · Phylogeny · Taxonomy

Introduction

The genus *Lepiota* (Pers.: Fr.) S.F. Gray, in the concept of Vellinga (2001a), includes the pale-spored members of the *Agaricaceae*, defined morphologically by the dextrinoid, non-metachromatic, and binucleate spores, absence of pleurocystidia, presence of cheilocystidia, regular hymenophoral trama, and presence of clamp-connections. Not all these characters are present in all species of the genus, e.g. *Lepiota fuscovinacea* J.E. Lange & F.H. Møller lacks clamp-connections and has uninucleate spores; *Lepiota cristatoides* Eihell. has non-dextrinoid and metachromatic spores (Vellinga 2001a). There is a wide variation in terms of spore shapes (fusiform, penguin-shaped, ellipsoid, spurred) and pileus covering structure (cutis, trichoderm, hymeniderm, chains of rounded elements), and these characters have played a major role in the infrageneric classification of *Lepiota* (Candusso and Lanzoni 1990; Bon 1993; Vellinga 2001a). The genus *Macrolepiota* Singer differs in the trabecular hymenophoral trama, the basidiospores provided with a germ-pore covered with an hyaline cap and the structure of the universal veil (Vellinga 2001a). Molecular data have supported this concept of *Lepiota*, but the position of the species with globose elements in the pileus covering (*Lepiota* section *Echinatae* Fayod) with respect to *Cystolepiota* Singer and *Melanophyllum* Velen., remains unresolved (Vellinga 2003, 2004).

The major mycofloristic works in the American tropics (Murrill 1911, 1914; Dennis 1952, 1961, 1970; Pegler 1983) have commonly used a much wider concept of *Lepiota*, that includes many species currently placed in the *Leucoagaricus/Leucocoprinus* clade (Vellinga 2003). There are some molecular data originating from Neotropical collections of lepiotaceous fungi, but most of them come from the studies

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on ant-associated fungi and their free-living relatives (Vo et al. 2009) that do not focus on taxonomy.

Over the past ten years, the second author (C.A.) has collected and studied fungi in the Dominican Republic. More than 300 species of macrofungi have been recorded, vouchered, and deposited in the herbarium of the Jardín Botánico Nacional Dr. Rafael Ma. Moscoso (JBSD-Santo Domingo, Dominican Republic). Approximately 20 % of the collections represent lepiotaceous fungi of different genera (*Chlorophyllum* Masee, *Cystolepiota*, *Lepiota*, *Leucoagaricus* Locq. ex Singer, *Leucocoprinus* Pat., *Smithiomyces* Singer) and are currently being studied and sequenced.

This contribution focuses on the description of two new species of *Lepiota* sensu stricto, *Lepiota squamulodiffracta* and *Lepiota sosuensis*, based on morphological and molecular (nuclear ribosomal Internal Transcribed Spacer region, nrITS) data. We also provide a full description and the first molecular data for *Lepiota subgranulosa* Murrill.

Materials and methods

Morphological study The basidiomata were photographed fresh in habitat using a digital camera Nikon coolpix 8400 and subsequently dried. Collections were studied using standard procedures for morphological examination of lepiotaceous fungi (Candusso and Lanzoni 1990; Vellinga 2001a). Microscopical observations were made on a Nikon Eclipse E200 microscope. Descriptive terms for morphological features follow Vellinga (1988, 2001a). The notation [35/2/1] indicates that measurements were made on 35 spores, in two samples, in one collection. At least 35 spores were measured per collection. Color codes are from Munsell Soil Color Charts (Munsell Color 2009). The following abbreviations are used in the descriptions: avl for average length, avw for average width, Q for quotient of length and width, and avQ for average quotient. Herbarium acronyms follow Thiers (2015).

DNA extraction, PCR, and sequencing Protocols for DNA extraction, PCR, and sequencing were the same as those outlined in Justo and Hibbett (2011). PCR amplification and sequencing of the nrITS region was performed using primers ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993). Raw sequence data were edited and assembled in Sequencher 4.7 (Gene Codes Corporation).

Phylogenetic analysis A total of 140 nrITS sequences were downloaded from GenBank, originally generated in the studies of Vellinga (2001b, c, 2003, 2007, 2010), Liang and Yang (2011a, b), Liang et al. (2011), Sysouphanthong et al. (2011, 2012), Birkebak et al. (2011), Lebel and Vellinga (2013), Ge and Smith (2013), Kropp et al. (2012), Razaq

Fig. 1 Best tree from the Maximum Likelihood analysis of the nrITS dataset of the genus *Lepiota*. Bootstrap values over 70 % are shown on or below the branches

et al. (2012), Vizzini et al. (2014a, b), Nawaz et al. (2013) and Caballero et al. (2015). This set of sequences includes a wide representation of the different lineages of *Lepiota* sensu Vellinga (2001a, 2003) with the exception of section *Echinatae*. The final dataset includes 143 *Lepiota* sequences, with *Macrolepiota procera* (Scop.: Fr.) Singer and *Macrolepiota rhodosperma* P.D. Orton as outgroup taxa. GenBank numbers and geographic origin for all sequences are given in Fig. 1. Sequences were aligned using MAFFT version 7 (Katoh and Toh 2008) and the strategy FFT-NS-i was selected. The alignment was inspected and manually corrected using AliView (Larsson 2014). A Maximum Likelihood analysis was run in the RAXML servers (Stamatakis et al. 2008), under a GTR model with 100 rapid bootstrap (BS) replicates.

Results

The final dataset consists of 143 ingroup sequences and a total of 843 characters (gaps included). The alignment file has been deposited in TreeBASE (S17540). The phylogenetic position of *Lepiota squamulodiffracta*, *L. sosuensis*, and *L. subgranulosa* is highlighted in Fig. 1.

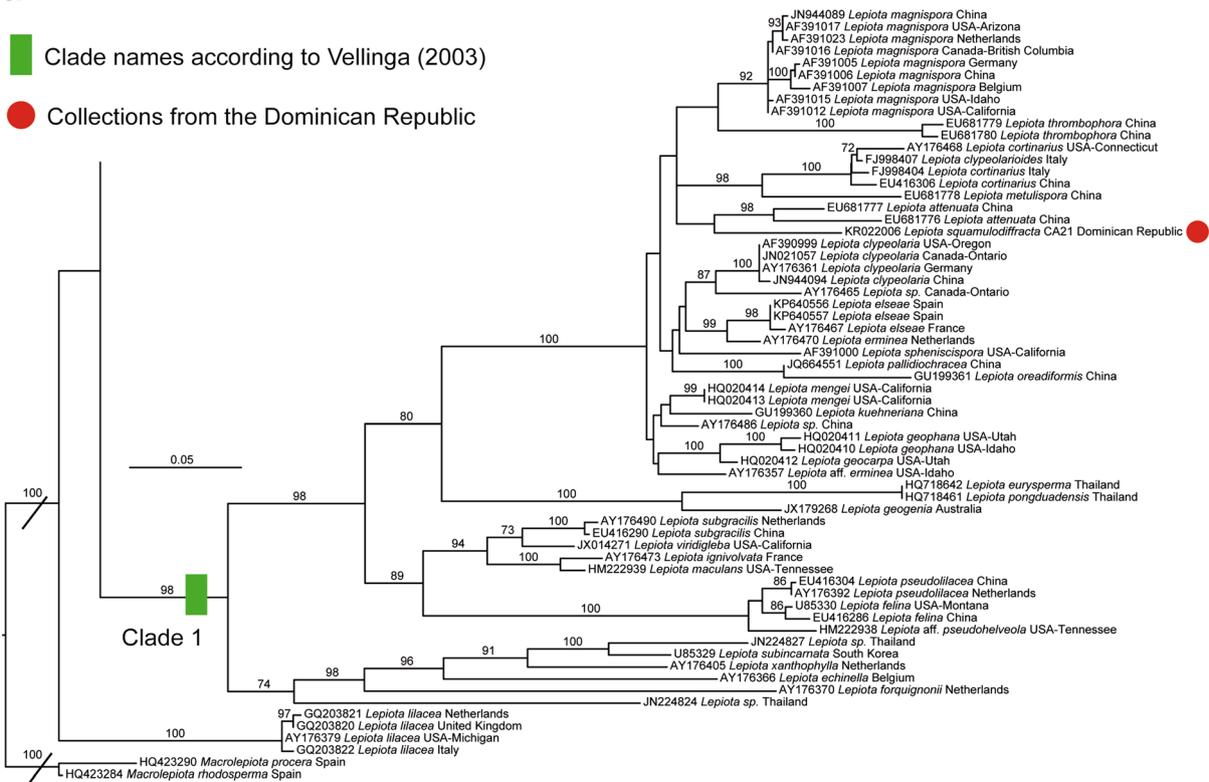
L. squamulodiffracta appears in the phylogenies in a well-supported (98 % BS) clade that corresponds to “Clade 1” in the phylogenies of Vellinga (2003). This clade includes all sampled representatives of section *Lepiota*, some elements of section *Ovisporae* (J.E. Lange) Kühner, and some gasteroid members of *Lepiota* such as *L. geogenia* T. Lebel & Vellinga, *L. geophana* (Kropp & Albee-Scott) Vellinga & T. Lebel, *L. geocarpa* (Kropp & L.J. Hutchison) Vellinga & T. Lebel, *L. mengei* (Kropp & Castellano) T. Lebel & Vellinga, and *L. viridigleba* (Castellano) Z. W. Ge & M. E. Sm. *Lepiota attenuata* J.F. Liang & Zhu L. Yang appears as sister to *L. squamulodiffracta*, but without support, and both taxa appear in a more inclusive clade with *L. magnispora* Murrill, *L. cortinarius* J.E. Lange, *Lepiota metulispora* (Berk & Broome) Sacc., and *L. thrombophora* (Berk. & Broome) Sacc., but again without support.

L. sosuensis and *L. subgranulosa* appear in the phylogenies in a well-supported (91 % BS) clade that corresponds to “Clade 2” in the phylogenies of Vellinga (2003). This clade includes representatives of sections *Stenosporae* (J.E. Lange) Kühner and *Ovisporae*. *L. sosuensis* appears in Clade 2a (Vellinga 2003) that does not appear supported in the analysis, as the sister species to *Lepiota vellingana* R.Nawaz & A. N. Khalid with 72 % BS, and both species appear in a well-supported (100 % BS) clade together with *Lepiota farinolens*

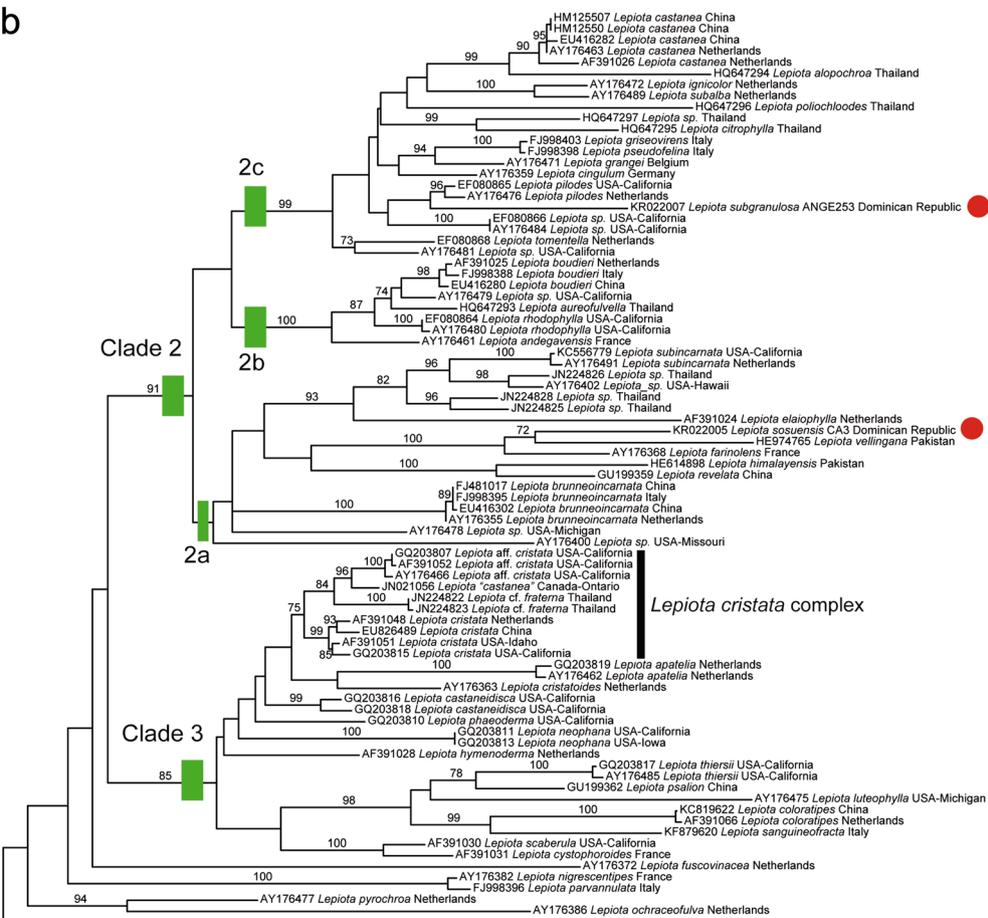
a

■ Clade names according to Vellinga (2003)

● Collections from the Dominican Republic



b



Bon & G. Rioussset. *Lepiota subgranulosa* appears in the well-supported (99 % BS) Clade 2c, as sister to *Lepiota pilodes* Vellinga & Huijser, but without support.

Taxonomy

***Lepiota squamulodiffracta* Justo, Bizzi & Angelini, sp. nov. (Fig. 2)**

Mycobank 812343

Diagnosis Similar to *L. metulispora*, differing in the more markedly striate-sulcate pileus, the velar patches on pileus split up into numerous minute squamules, basidiospores with higher avQ values, longer pileus covering elements and different nrITS sequence.

Holotype Dominican Republic. Prov. Puerto Plata: Sosúa, Puerto Chiquito, 25 Nov. 2011, coll. CA 21, nrITS KR022006 (JBSD, isotype at MEXU)

Etymology The name is a combination of the Latin “squamula” (small scale) and “diffracta” (shattered, broken into pieces). It is given to this species for the aspect and distribution of the squamules on the pileus surface.

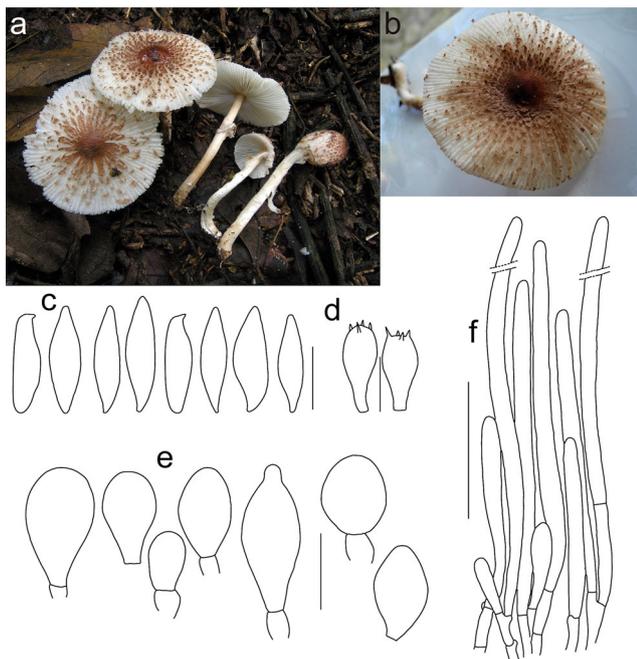


Fig. 2 *Lepiota squamulodiffracta*. **a** Basidiocarps (coll. CA21); **b** Basidiocarps (coll. ANGE245); **c** Basidiospores (scale bar=10 μ m); **d** Basidia (scale bar=20 μ m); **e** Cheilocystidia (scale bar=20 μ m); **f** Pileus covering (scale bar=100 μ m). All microscopic characters from coll. CA21

Pileus Diameter 50–60 mm, hemispherical or conico-convex with rounded apex when young, expanding to appanate with distinct to low and broad umbo; surface strongly striate or striate-sulcate up to the center of pileus, white and radially fibrillose, set with brown, red brown, or orange brown (Mu. 5YR 6/8, 5/8; 2.5YR 6/8) fibrillose squamules, radially arranged and slightly uplifted; the center is slightly darker (Mu. 2.5YR 5/8, 4/8; 5YR 4/6) and does not break up into squames; margin strongly striate or striate sulcate, with few or no velar remnants. **Lamellae** moderately crowded to distant, free, ventricose, 3–5 mm wide, white, with some yellow tones in damaged or old specimens; with entire to fimbriate, white edges. **Stipe** 70–100 \times 4–5 mm, cylindrical to slightly widened at base, often curved at the base, in upper part white to cream, sometimes with red-brown tints, smooth to innately lengthwise fibrillose; with a fibrillose annular zone, situated halfway to one third from apex; annulus white decorated with brown squamules, fragile and evanescent; below the annular zone, surface more conspicuously fibrillose, without floccose velar remnants, turning slightly yellow or yellow-orange if scratched; at the base sometimes with small squamules similar to those on pileus. **Context** in pileus and stipe white. **Smell** unpleasant, like the rubber component in the smell of *Lepiota cristata* (Bolton: Fr.) P. Kumm.; **taste** similar to smell.

Basidiospores [70/2/2] 14.0–19.0(19.5) \times 4.0–6.5 μ m, avl \times avw=15.3–17.7 \times 4.3–5.7 μ m, Q=2.4–4.2, avQ=3.3, in side-view fusiform with more or less straight abaxial side (“penguin-shaped”), in frontal-view fusiform, with suprahilar depression, dextrinoid, not metachromatic in Cresyl Blue. **Basidia** 19–34.5 \times 6–12 μ m, clavate, 4-spored. **Pleurocystidia** absent. **Cheilocystidia** 13–34 \times 9–17 μ m, clavate or broadly clavate, a few utriform, thin-walled and colorless. **Pileus covering** a trichoderm, made up of cylindrical elements 145–575 \times 5–11.5 μ m, slightly attenuated at apex, without internal septa or more rarely with an internal, non-clamped septum in the lower part; in between the longer elements there is an irregular layer of shorter cylindrical or cylindrical-clavate elements; pigment parietal, brown or yellow-brown. **Stipe covering** a cutis, made up of cylindrical hyphae, 5.0–8.5 μ m wide, mostly hyaline. **Clamp-connections** present.

Habit, habitat, and phenology Gregarious, fruiting in groups of up to seven specimens. On the litter layer of broad-leaved forests, with strong anthropogenic influence. November.

Distribution Known from one locality in the Dominican Republic (Puerto Plata: Sosúa, Puerto Chiquito)

Material examined Dominican Republic. Prov. Puerto Plata: Sosúa, Puerto Chiquito, 25 Nov. 2011, coll. CA 21; *ibidem*, 21 Nov. 2013, coll. ANGE245.

Observations The brown to red brown, strongly dissociated squames on the pileus, and the striate-sulcate surface up to the pileus center are the most distinctive external characters of *Lepiota squamulodiffracta*. The species that appear closest to the new taxon in the nrITS phylogenies (*L. attenuata*, *L. magnispora*, *L. cortinarius*, *L. metulispora*, and *L. thrombophora*; Fig. 1a) share the penguin-shaped spores, i.e. fusiform with a more or less straight abaxial side (Pegler 1986; Vellinga 2001a, b; Liang et al. 2011). Macroscopically all these taxa, except *L. metulispora*, lack a strongly sulcate-striate pileus surface, and are only sulcate-striate at the margin or not at all. *L. metulispora* comes close to *L. squamulodiffracta* in the fact that the pileus can be strongly sulcate-striate, but differs in the slightly smaller basidiocarps (pileus up to 40 mm), the comparatively broader spores (avQ=2.76–3.10 according to Liang et al. 2011 and Sysouphanthong et al. 2012), and the shorter pileus covering elements, up to 230 μm long (Pegler 1986; Liang et al. 2011; Sysouphanthong et al. 2012). Based on nrITS data all the species mentioned above are clearly different from *L. squamulodiffracta*, with the most similar sequence (*L. attenuata*) being only 93 % identical. *Lepiota clypeolaria* f. *umbrinisquamosa* Hongo (syn. *L. ventriosospora* var. *umbrinosquamosa* (Hongo) Bon), is reminiscent of *L. squamulodiffracta* in the striate-sulcate margin of pileus, but the striations do not reach the pileus center (Hongo 1970). This taxon has been subsumed in the synonymy of *L. magnispora* by Vellinga (2001b).

There are few species morphologically assignable to section *Lepiota* that have been described or reported from the Caribbean area. Pegler (1983) did not record any species of the section from the Lesser Antilles. Akers and Sundberg (2000) reported *Lepiota clypeolaria* (Bull.: Fr.) P. Kumm. and *L. aspericeps* Murrill from Florida (USA). *L. clypeolaria* differs morphologically from *L. squamulodiffracta* in the velar patches on pileus covering much space and showing less background, generally paler colors, the non-strongly striate-sulcate pileus surface on mature specimens, the presence of wooly velar remnants on the stipe, and the basidiospores with convex abaxial and adaxial sides. Molecularly, both species are also clearly distinct (Fig. 1). *Lepiota aspericeps* has smaller pilei (up to 30 mm) with paler squamules, a membranous persistent ring on the stipe, and comparatively smaller basidiospores (8.2–13 \times 4.1–5.2 μm , Q=2.1) that are not penguin-shaped. *Lepiota mississippiensis* Murrill, originally described from the southern USA, might belong to section *Lepiota* on account of the general aspect of the basidiocarps, the fusiform spores and the long, erect elements on the pileus covering (Murrill 1914; Smith 1966), however Smith (1966) did not observe clamp-connections on her study of the type collection. Additional types studies did not reveal the presence of clamp-connections either (<http://sweetgum.nybg.org/vh/specimen.php?irn=816404>). *L. mississippiensis* differs from *L.*

squamulodiffracta in the much smaller basidiocarps (pileus up to 20 mm), the pileus only faintly striate at margin, and the smaller, non penguin-shaped, basidiospores “11–14(16) \times 4.4–6 μm ”, according to Smith (1966). *Lepiota maculans* Murrill, originally described from Missouri, but recently discovered in the Great Smoky Mountains National Park (Tennessee/North Carolina, USA) also belongs to this section, but differs in the salmon discoloration of the basidiomata (Birkebak et al. 2011).

Among the Caribbean species reported by Dennis (1952, 1961, 1970), *Lepiota floralis* (Berk. & Ravenel) Sacc. also has brown to orange-brown squamules on the pileus, but they are much smaller and not strongly dissociated, the pileus is not striate-sulcate, the stipe is brown to orange-brown, and the basidiospores are much smaller, “6–8 (10.5) \times 2.75–3.5 (4) μm ”, according to Dennis (1952). *Lepiota zamurensis* Pat. & Gaillard has a strongly sulcate pileus, sometimes up to the disk, but differs from *L. squamulodiffracta* in the grey-brown colors of the basidiocarps and the much smaller spores of 4–5 \times 2.75–3 μm (Dennis 1952, 1970). *Lepiota bakeri* Dennis, has minute orange-brown squamules on pileus and the pileus margin may appear sulcate, but the basidiospores are much smaller, 5–7 \times 3.5–4 μm (Dennis 1952, 1970).

Other tropical representatives of section *Lepiota* from Asia include *L. euryserma* Sysouphanthong, K.D. Hyde & Vellinga, with much smaller (7.8–11.5 \times 4.5–6.5 μm) and non-penguin-shaped spores (Sysouphanthong et al. 2012); *L. microcarpa* Sysouphanthong, K.D. Hyde & Vellinga, with pileus up to 6 mm in diameter (Sysouphanthong et al. 2012); and *L. pongduadensis* Sysouphanthong, K.D. Hyde & Vellinga, with distinctly radial streaks of brown squamules and a white fibrillose or floccose stipe with squamules (Sysouphanthong et al. 2012).

Lepiota sosuensis Justo, Bizzi & Angelini, sp. nov. (Fig. 3)

Mycobank 812344

Diagnosis Similar to *Lepiota vellingana*, differing in the non-markedly squamose pileus, the elements of the pileus covering without clamp-connections, and different nrITS sequence.

Holotype Dominican Republic. Prov. Puerto Plata: Sosúa, Puerto Chiquito, 28 Dec. 2010, coll. CA 3, nrITS KR022005 (JBSD, isotype at MEXU)

Etymology The name comes from the type locality (Sosúa).

Pileus Diameter 25–55 mm, hemispherical when young, then convex to plano-convex with a broad central umbo; surface when young with minute fibrillose squamules, at maturity becoming overall radially fibrillose, without discrete squamules; white, sometimes with pale yellow-brown (Mu.

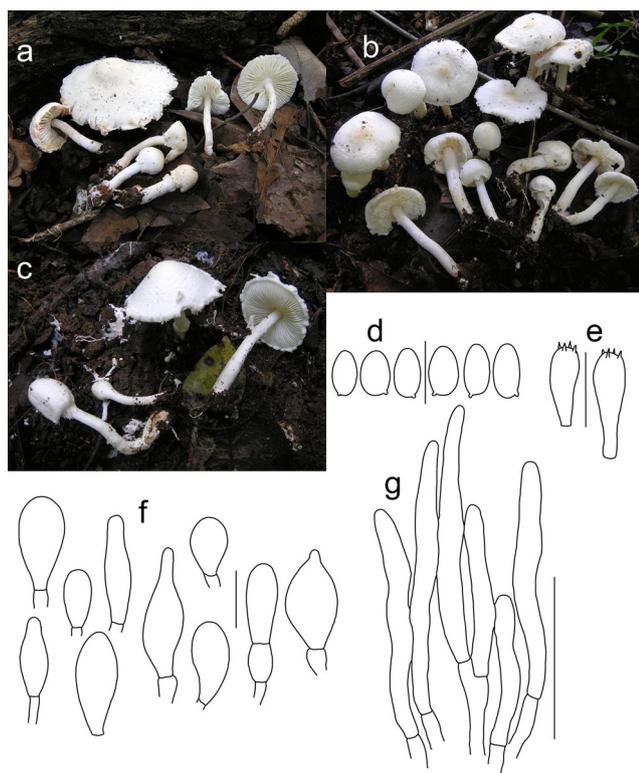


Fig. 3 *Lepiota sosuensis*. **a** Basidiocarps (coll. CA3), **b** Basidiocarps (coll. ANGE240); **c** Basidiocarps (coll. ANGE499); **d** Basidiospores (scale bar=10 μm); **e** Basidia (scale bar=20 μm); **f** Cheilocystidia (scale bar=20 μm); **g** Pileus covering (scale bar=100 μm). All microscopic characters from coll. CA3

10YR 8/3, 8/4; 2.5Y 8/3, 8/4) or pale-pink, cream-pink (Mu. 2.5YR 8/2, 8/3) tints at center or around margin, especially in older basidiocarps; margin entire, appendiculate with fibrillose, white or white-cream remnants, commonly triangularly shaped. **Lamellae** moderately crowded to slightly distant, free, ventricose, 2–4 mm wide, white; with entire to fimbriate, white edges. **Stipe** 30–40 \times 4–7 mm, cylindrical to slightly widened at base, in upper part white to pale cream, smooth; with a fibrillose annular zone, situated halfway to one third from apex, conspicuous in young specimens, then breaking up and leaving velar remnants the pileus margin and on the stipe surface; below the annular zone, surface more conspicuously and densely fibrillose, white, sometimes with some pink tones near the base. **Context** in pileus and stipe white, unchanging. **Smell** unpleasant, like the rubber component in the smell of *Lepiota cristata*, **taste** similar to smell, bitter.

Basidiospores [140/7/4] (6.0)6.5–9.0 \times 3.5–5.0 μm , avl \times avw=6.7–7.9 \times 3.9–4.5 μm , Q=1.4–2.1, avQ=1.8, ellipsoid to oblong, ovoid, dextrinoid, with pink inner wall (metachromatic) in Cresyl Blue. **Basidia** 17–34 \times 7–11 μm , clavate, 4-spored. **Pleurocystidia** absent. **Cheilocystidia** 14–48 \times 6–16 μm , clavate or broadly clavate, (narrowly) utriform or cylindrical, thin-walled and colorless. **Pileus**

covering a trichoderm, made up of cylindrical elements 30–237 \times 6.5–12.5 μm , slightly attenuated at apex, with internal, non-clamped septa, colourless. **Stipe covering** made up of cylindrical elements, similar to those of pileus but shorter, 40–105 \times 7.5–11 μm . **Clamp-connections** present but rare, only observed at the base of some basidia, basidioles, and cheilocystidia in the hymenium.

Habit, habitat, and phenology Gregarious, fruiting in groups of up to 15–20 specimens. On the litter layer of broad-leaved forests, with strong anthropogenic influence. Commonly fruiting in very humid zones (i.e near small streams). December.

Distribution Known from one locality in the Dominican Republic (Puerto Plata: Sosúa, Puerto Chiquito)

Material examined Dominican Republic. Prov. Puerto Plata. Sosúa, Puerto Chiquito 28 Dec. 2010, coll. CA3; *ibidem* 30 Nov. 2013 coll. ANGE 240 and ANGE241; *ibidem* 30 Dec. 2014, coll. ANGE499; *ibidem* 20 Dec. 2014, coll. ANGE506

Observations In the phylogenetic analysis *L. sosuensis* appears as sister to *Lepiota vellingana* (Fig. 1b). Both taxa share the overall white basidiocarps with pinkish tones, but *L. vellingana* differs from *L. sosuensis* in the more markedly squamose pileus and the clamped terminal elements of the pileus covering (Nawaz et al. 2013). In *L. sosuensis* clamp-connections were not observed on the pileus covering, only at the base of some elements of the hymenium. The spores of *L. sosuensis* show a metachromatic reaction in cresyl blue, but unfortunately in the description of *L. vellingana* this character is not mentioned (Nawaz et al. 2013). The nrITS sequences of both species are only 87 % identical.

Other species of *Lepiota* s. stricto with slightly to strongly metachromatic spores, such as *L. apatelia* Vellinga & Huijser, *L. castaneidisca* Murrill, *L. cristatoides*, *L. hymenoderma* D.A. Reid, *L. luteophylla* Sundb., *L. neophana* Morgan, *L. phaeoderma* Vellinga, *L. scaberula* Vellinga, and *L. thiersii* Sundb., have a hymeniform pileus covering and appear concentrated in Clade 3 (Fig. 1b), while *Lepiota lilacea*, also with metachromatic spores and hymeniform pileus covering, takes an isolated position in the phylogenies (Fig. 1a). For a complete morphological and molecular overview of this group see Vellinga (2001a, 2010).

Lepiota farinolens Bon & G. Rioussset, which also appears close to *L. sosuensis* in the phylogeny (Fig. 1b), is very different macroscopically, with overall brown or brown-pink colors of the basidiocarps and a strong farinaceous smell (Salom and Siquier 2001; Vellinga 2001a).

The clade containing *L. sosuensis* harbors the deadly, amantitin-containing, species of *Lepiota* (*L. brunneoincarnata*

Chodat & C. Martin, *L. subincarnata* J. E. Lange, *L. elaiophylla* Vellinga & Huijser), but it is not known if all taxa in the clade contain the same toxins.

***Lepiota subgranulosa* Murrill, Mycologia 3: 83. 1911.**
(Fig. 4)

Pileus Diameter 10–15 mm, hemispherical when young, then convex to plano-convex with a broad, prominent, central umbo; surface covered with minute, scurfy squamules, yellow-brown (Mu. 10YR 7/8, 8/6, 8/8; 2.5Y 8/6, 8/8), darker towards center (Mu. 7.5YR 5/8, 6/8, 7/8); margin entire, appendiculate with squamulose veil remnants. **Lamellae** moderately crowded, free, ventricose, 1–2 mm wide, white; with entire to fimbriate, white edges. **Stipe** 20–40×2–3 mm, cylindrical, in upper part white to cream, fibrillose; with an annular zone, about one third from apex, evanescent, yellow-brown, with the margin more intensely colored; below the annular zone, surface conspicuously covered with squamules similar to those on pileus. **Context** in pileus and stipe white, unchanging. **Odor** indistinct, **taste** not recorded.

Basidiospores [35/1/1] 5.0–6.5×2.5–3.5 μm , $\text{avl}\times\text{avw}=5.9\times 3.0$ μm , $Q=1.7\text{--}2.6$, $\text{av}Q=2.0$, oblong to cylindrical with a spurred base, often with a swelling on the abaxial side, or several swellings that may be on both abaxial and adaxial sides, giving the spores a bumpy, irregular outline, dextrinoid,

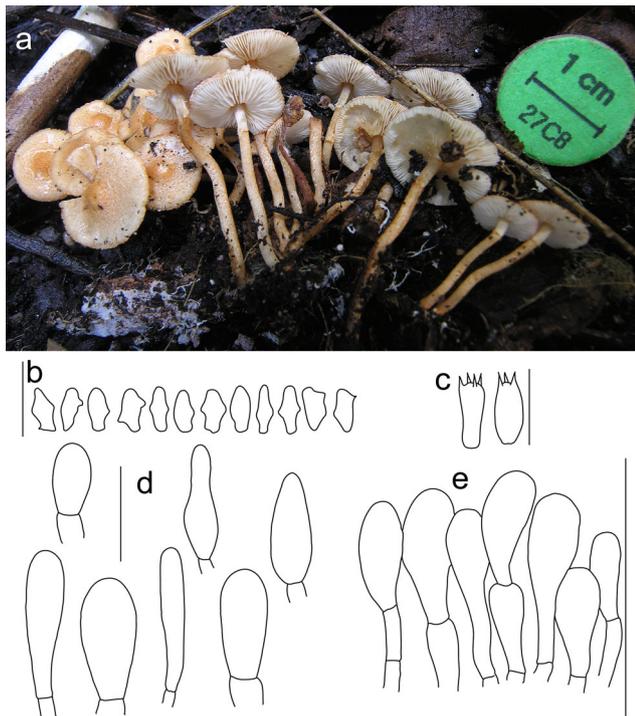


Fig. 4 *Lepiota subgranulosa*. **a** Basidiocarps; **b** Basidiospores (scale bar=10 μm); **c** Basidia (scale bar=20 μm); **d** Cheilocystidia (scale bar=20 μm); **e** Pileus covering (scale bar=100 μm). All from coll. ANGE253

non metachromatic in Cresyl Blue. **Basidia** 14–19×6–8 μm , clavate, 4-spored. **Pleurocystidia** absent. **Cheilocystidia** 13.5–32×5–12 μm , narrowly clavate or cylindrical, a few narrowly utriform, thin-walled and colorless. **Pileus** covering a hymeniderm or irregular hymeniderm, made up of clavate elements 27–65×10–17.5 μm , slightly thick-walled, with parietal, yellow-brown pigment. **Stipe covering** made up of elements similar to those on pileus. **Clamp-connections** present but rare, observed in the pileus covering.

Habit, habitat, and phenology Gregarious fruiting in groups of up to 30 specimens. The Dominican collection was made on the litter layer of broad-leaved forests, with strong anthropogenic influence; also recorded growing on soil in a banana plantation, on wood and in a coffee plantation (Murrill 1911; Dennis 1952; Pegler 1986). November in the Dominican Republic; September–November in the Lesser Antilles (Dennis 1952; Pegler 1986); January in Mexico (Murrill 1911).

Distribution Known from one locality in the Dominican Republic (Puerto Plata: Sosúa, Puerto Chiquito). Also known from Trinidad and Martinique in the Lesser Antilles (Dennis 1952; Pegler 1986) and Mexico (Murrill 1911).

Material examined Dominican Republic. Prov. Puerto Plata: Sosúa, Puerto Chiquito, 30 Nov. 2013, coll. ANGE 253 (JBSD, duplicate in MEXU).

Observations Murrill (1911) originally described the spores of *L. subgranulosa* as “ellipsoid”; however, both Dennis (1952) and Pegler (1983), who studied the type collection, described the spores as spurred and with a protuberance on the abaxial side. Our observations agree with Dennis (1952) and Pegler (1983), although the protuberances can be also on the adaxial side, giving the spores a bumpy and irregular outline. Morphologically, our collection agrees well with the description of Pegler (1983). *Lepiota pseudoignicolor* Dennis, originally described as *L. subgranulosa* var. *majus* Dennis, differs in the bigger basidiocarps (pileus up to 30 mm) and the bigger basidiospores, 8–11×3–4 μm (Dennis 1952, 1961).

There are several species of *Lepiota* with somewhat similar basidiospores, spurred, and with obvious lateral knobs on abaxial and adaxial sides: (i) *Lepiota fraternata* Horak, described from Papua New-Guinea, differs in the red-brown colors of the pileus, the membranous, ascendant ring on the stipe, and the smaller elements of the pileus covering, 28–40×6–10 μm (Horak 1980); (ii) *Lepiota aurora* Horak (nom. illeg., non *L. aurora* Murrill), also described from Papua-New Guinea differs in the orange to red-orange basidiocarps and the bigger basidiospores of 6–8.5×3–4.5 μm (Horak 1980); (iii) *Lepiota cristata* var. *macrospora* (Zhu L. Yang) J.F. Liang & Zhu L. Yang differs in the bigger basidiocarps (pileus up to 50 mm),

and the non-dextrinoid and metachromatic basidiospores (Liang and Yang 2011a). The phylogenetic analyses of Liang et al. (2009) place *L. cristata* var. *macrospora* as part of the *L. cristata* species complex. This group of taxa appears in our phylogenies as part of Clade 3, not closely related to *L. subgranulosa* (Fig. 1b). Two collections from Thailand tentatively identified as *L. fraterna* also fall in the *L. cristata* complex (Fig. 1b; Sysouphanthong et al. 2011). There are no molecular data of *Lepiota aurora* available for comparison.

Phylogenetically *L. subgranulosa* belongs in clade 2c (Fig. 1b), together with species with spurred spores and a trichodermial pileus covering (Vellinga 2003). In addition to the irregular-looking spores, *L. subgranulosa* also stands out morphologically from the species in this clade by the hymeniform pileus covering, with clavate-pedunculate elements not more than 65 µm long. All species in this group sampled for molecular data have a trichodermial pileus covering, with elements in all cases more than 100 µm long, and in temperate taxa easily reaching 200 or 300 µm long (see Vellinga 2001a for full descriptions of temperate taxa). The three tropical taxa, for which molecular data are available, have comparatively shorter elements of the pileus covering than the temperate taxa: *L. alopochroa* (Berk & Broome) Sacc. 50–113 µm long, *L. citrophylla* (Berk & Broome) Sacc. 50–160 µm long, *Lepiota* sp. (HQ647297) 50–170 µm long (Sysouphanthong et al. 2011). In all cases these pileus covering elements are still much longer than in *L. subgranulosa*.

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References

- Akers BP, Sundberg WJ (2000) *Lepiotaceae* of Florida, III. *Lepiota* s.str., section *Lepiota*. Mycotaxon 75:137–146
- Birkebak JM, Vellinga EC, Franco-Molano AE, Wood MG, Matheny PB (2011) *Lepiota maculans*, an unusual mushroom rediscovered after 105 years. Southeast Nat 10:267–274. doi:10.1656/058.010.0207
- Bon M (1993) Flore Mycologique d'Europe 3: Les Lepiotes. Association d'Ecologie et de Mycologie, Lille
- Caballero A, Vizzini A, Muñoz G, Contu M, Ercole E (2015) *Lepiota elseae* (Agaricales, Agaricaceae), a new species of section *Lepiota* from Spain. Phytotaxa 201:188–196. doi:10.11646/phytotaxa.201.3.2
- Candusso M, Lanzoni G (1990) *Lepiota* s.l. Fungi Europaei 4. Giovanna Bella, Saronno, Italy
- Dennis RWG (1952) *Lepiota* and allied genera in Trinidad, British West Indies. Kew Bull 7:459–499
- Dennis RWG (1961) Fungi venezuelani. IV. Agaricales. Kew Bull 15:67–156
- Dennis RWG (1970) Fungus Flora of Venezuela and adjacent countries. Kew Bull Addit Ser 3:1–531
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Mol Ecol 2:132–118
- Ge ZW, Smith ME (2013) Phylogenetic analysis of rDNA sequences indicates that the sequestrate *Amogaster viridiglebus* is derived from within the agaricoid genus *Lepiota* (Agaricaceae). Mycol Prog 12: 151–155. doi:10.1007/s11557-012-0841-y
- Hongo T (1970) Notulae Mycologicae 9. Memoirs Shiga University 20: 49–54
- Horak E (1980) On Australasian species of *Lepiota* S.F. Gray (Agaricales) with spurred spores. Sydowia 33:111–144
- Justo A, Hibbett DS (2011) Phylogenetic classification of *Trametes* (Basidiomycota, Polyporales) based on a five-marker dataset. Taxon 60:1567–1583
- Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform 9:286–298. doi:10.1093/bib/bbn013
- Kropp BR, Albee-Scott S, Castellano M, Trappe JM (2012) *Cryptolepiota*, a new sequestrate genus in the Agaricaceae with evidence for adaptive radiation in western North America. Mycologia 104:164–174. doi:10.3852/11-046
- Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30:3276–3278. doi:10.1093/bioinformatics/btu531
- Lebel T, Vellinga EC (2013) Description and affinities of a sequestrate *Lepiota* (Agaricaceae) from Australia. Mycol Prog 12:525–532. doi: 10.1007/s11557-012-0859-1
- Liang JF, Yang ZL (2011a) Two new taxa close to *Lepiota cristata* from China. Mycotaxon 116:387–394. doi:10.5248/116.387
- Liang JF, Yang ZL (2011b) A new species of *Lepiota* (Agaricaceae) from southwestern China. Mycotaxon 117:359–363. doi:10.5248/117.359
- Liang JF, Xu DP, Yang ZL (2009) Divergence, dispersal and recombination in *Lepiota cristata* from China. Fung Div 38:105–124
- Liang JF, Yang ZL, Xu DP (2011) A new species of *Lepiota* from China. Mycologia 103:820–830. doi:10.3852/10-216
- Munsell Color (ed) (2009) Munsell Soil-Color Charts. Grand Rapids, Michigan (U.S.A.)
- Murrill WA (1911) The Agaricaceae of tropical North America II. Mycologia 3:79–91
- Murrill WA (1914) Agaricaceae. North Am Flora 10:41–65
- Nawaz R, Khalid AN, Hanif M, Razaq A (2013) *Lepiota vellingana* sp. nov. (Basidiomycota, Agaricales) a new species from Lahore, Pakistan. Mycol Prog 12:727–732. doi:10.1007/s11557-012-0884-0
- Pegler DN (1983) Agaric Flora of the Lesser Antilles. Kew Bull Addit Ser 9:1–668
- Pegler DN (1986) Agaric Flora of Sri Lanka. Kew Bull Addit Ser 12:1–519
- Razaq A, Khalid AN, Vellinga EC (2012) *Lepiota himalayensis* (Basidiomycota, Agaricales), a new species from Pakistan. Mycotaxon 121:319–325. doi:10.5248/121.319
- Salom JC, Siquier JL (2001) Contribució al coneixement de la Família Lepiotaceae Roze a les Illes Balears. II. Rev Catalana Micol 23:109–120
- Smith HV (1966) Contributions toward a monograph on the genus *Lepiota*, I. Type studies in the genus *Lepiota*. Mycopath Mycol appl 29:97–117
- Stamatakis A, Hoover P, Rougemont J (2008) A Rapid Bootstrap Algorithm for the RAxML Web-Servers. Syst Biol 57:758–771
- Sysouphanthong P, Hyde KD, Chuksatiro E, Bahkali AH, Vellinga EC (2011) *Lepiota* (Agaricales) in northern Thailand – 1. *L.* section *Stenosporae*. Mycotaxon 117:53–85. doi:10.5248/117.53

- Sysouphanthong P, Hyde KD, Chukeatirote E, Bahkali AH, Vellinga EC (2012) *Lepiota* (Agaricales) in northern Thailand-2 *Lepiota* section *Lepiota*. Cryptog Mycol 33:25–42
- Thiers B (2015) Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Gardens's Virtual Herbarium (continuously updated). <http://sweetgum.nybg.org/ih/>
- Vellinga EC (1988) Glossary. In: Bas C, Kuyper TW, Noordeloos ME, Vellinga EC (eds) Flora Agaricina Neerlandica, vol 1. A.A. Balkema, Rotterdam, pp 54–64
- Vellinga EC (2001a) *Lepiota*. In: Noordeloos ME, Kuyper TW, Vellinga EC (eds) Flora Agaricina Neerlandica 5. A.A. Balkema, Lisse, pp 109–151
- Vellinga EC (2001b) Studies in *Lepiota* III. Some species from California, U.S.A. Mycotaxon 80:285–296
- Vellinga EC (2001c) Studies in *Lepiota* IV. *Lepiota cristata* and *L. castaneidisca*. Mycotaxon 80:297–306
- 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 (2007) Lepiotaceous fungi in California, U.S.A. – 2. *Lepiota rhodophylla*. Mycotaxon 98:205–211
- Vellinga EC (2010) *Lepiota* in California: species with a hymeniform pileus covering. Mycologia 102:664–674. doi:10.3852/09-180
- Vizzini Ercole E, Voyron S (2014) *Lepiota sanguineofracta* (Basidiomycota, Agaricales), a new species with a hymeniform pileus covering from Italy. Mycol Prog 13:683–690
- Vizzini A, Liang JF, Jančovičová S, Adamčík S, Ercole E, Contu M, Yang ZL, Vellinga EC (2014) *Lepiota coloratipes*, a new species for *Lepiota rufipes* ss. Auct.europ. non ss. orig. Mycol Prog 13:171–179. doi:10.1007/s11557-013-0905-7
- Vo T, Mueller UG, Mikheyev AS (2009) Free-living fungal symbionts (Lepiotaceae) of fungus-growing ants (Attini: Formicidae). Mycologia 101:206–210. doi:10.3852/07-055
- White TJ, Bruns T, Lee SS, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp 315–322