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Author(s): Joshua M. Birkebak, Else C. Vellinga, Ana E. Franco-Molano, Michael G. Wood and P. Brandon Matheny

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Lepiota maculans, an Unusual Mushroom Rediscovered after 105 years

Joshua M. Birkebak^{1,*}, Else C. Vellinga², Ana E. Franco-Molano³,
Michael G. Wood⁴, and P. Brandon Matheny¹

Abstract - During a survey of macrofungi in the Great Smoky Mountains National Park in the summer of 2009, a species of *Lepiota* was recorded. This specimen is distinct in having pink to salmon to orange colors on the fruiting body and a pink spore deposit. After comparison with type material and a survey of the literature, we conclude this species is *L. maculans* Peck, described originally and known only from one collection in Missouri. Phylogenetic analysis of DNA sequence data supports placement within *Lepiota* of the Agaricaceae despite possessing a pink spore deposit. A complete morphological description, a description of the holotype, illustrations, and photographs are presented. This is the first report of *L. maculans* since its original description in 1905.

Introduction

Lepiota (Agaricaceae, Agaricales) is a large genus (estimated to have at least 500 species worldwide) of pale-spored, saprotrophic fungi that are poorly known in North America (Vellinga 2004). Most monographic treatments of the group (Kauffman 1924; Morgan 1906a, 1906b, 1906c, 1907; Murrill 1914) are outdated as they lacked information on many pertinent (particularly microscopic) details, and only one has focused on a portion of the southeastern United States (Akers 1997).

During a macrofungal diversity survey of the Great Smoky Mountains National Park by researchers affiliated with the University of Tennessee, University of California at Berkeley, University of Washington, Mycological Society of San Francisco, and British Mycological Society, a distinct *Lepiota* specimen was found with a predominantly pink and orange coloration. After further study, the identity was determined to be *Lepiota maculans* Peck, originally published from a collection made in Missouri (Peck 1905). To our knowledge, this fungus has not been reported since its original publication. A cursory examination of 79 undetermined specimens of *Lepiota* at the University of Tennessee herbarium was conducted in search of other collections of this species. Despite the extensive collection of fungi from the Great Smoky Mountains National Park (Hesler 1937, Lickey et al. 2007, Petersen 1979), no additional specimens of *L. maculans* were found.

¹Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996. ²Department of Plant and Microbial Biology, University of California, 111 Koshland Hall, Berkeley, CA 94720-3102. ³Laboratorio de Taxonomía de Hongos, Instituto de Biología, Universidad de Antioquia A.A.1226, Medellín, Colombia. ⁴14856 Saturn Drive, San Leandro, CA 94578-1349. *Corresponding author - jbirkeba@utk.edu.

Methods

Morphological examination

Color notations of fruitbodies were taken from Ridgway (1912). Microscopic characters were examined from dried material revived in 5% KOH. Spores for measurement were taken from the spore deposit, and 20 cells or spores were measured for each character. All measurement summaries are given in the following format: minimum–mean–maximum value. A Q-value is the ratio of cell/spore length to cell/spore width.

Molecular examination

DNA extraction. A dried tissue sample between 10–20 mg was excised and ground in liquid nitrogen with a micropestle and a pinch of sand in a 1.5-ml microtube. DNA extraction was performed initially with an E.Z.N.A.[®] Fungal DNA Kit (Omega Bio-Tek, Norcross, GA). This procedure yielded no PCR products so a high-performance kit, E.Z.N.A.[®] HP Fungal DNA Kit (Omega Bio-Tek), was used to remove polysaccharides and other potential interfering factors. The isolated genomic DNA was diluted in two successive 1:10 sterile-water dilutions.

PCR. Primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the ITS1-5.8S-ITS2 region (a commonly used barcode region in molecular fungal systematics) on a Bio-Rad C1000 thermal cycler (Bio-Rad, Hercules, CA). A mixture of sterile water and 5X buffer, GoTaq, and dNTPs supplied by Invitrogen Corp (Carlsbad, CA) was prepared for each dilution of DNA and controls following manufacturer protocols. Our PCR protocol followed that of White et al. (1990). PCR products were visualized on a 1.0% agarose gel prepared with ethidium-bromide and a UV transilluminator. The amplified ITS product of *L. maculans* was cleaned using a QIAquick PCR purification kit (Qiagen, Valencia, CA).

Sequencing. A sequence reaction was performed on the purified ITS product using a BigDye Terminator 3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA). The sequence reaction solution was purified with a Sephadex G-50 column (General Electric Healthcare, Piscataway, NJ) using separator strips manufactured by Princeton Separations (Freehold, NJ). Sequencing was performed on an ABI 3730 48-capillary electrophoresis genetic analyzer (Applied Biosystems) at the Molecular Biology Resource Facility at the University of Tennessee. Sequence chromatograms were inspected and edited using Sequencher 4.9 software (Gene Codes Corp, Ann Arbor, MI).

Phylogenetic analysis. The nrITS sequence was compared with those already present in GenBank using the program BLAST (Altschul et al. 1990), and based on the outcome a database of sequences from *Lepiota* sect. *Lepiota* was compiled. The nrITS sequences were aligned with the program MAFFT version 6 (Kato and Toh 2008, Kato et al. 2002). The sequence data matrix was analyzed by maximum likelihood (ML) using RAxML version 7.2.3 (Stamatakis et al. 2008). 100 rapid ML bootstraps were performed. *Lepiota ochraceofulva* P.D. Orton and *L. elaiophylla* Vellinga & Huijser were chosen as outgroup species. These species

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differ from the subject taxon by having non-fusiform spores and a different type
of pileus covering.

Results

Taxonomy

Lepiota maculans Peck, Bulletin of the Torrey Botanical Club 32(2):77 (1905) (Fig. 1).

Pileus. 3.2–4.0 cm broad, plano-convex often with a slight umbo; margin de-curved at first, becoming straight with expansion, somewhat sulcate striate, often eroded with age; disc “Dresden Brown”, “Ochraceous-Tawny” to “Buckthorn Brown”, velutinous, breaking up in scales near center of pileus, scales sparse, exposing context; context “Mustard Yellow” to “Amber Yellow” near the center, “Salmon-Buff” to “Light Salmon-Buff” to “Orange-Pink” outward, at very edge discolored “Peach Red” to “Scarlet”.

Lamellae. Free, nearly crowded, thin, 4–6 mm broad, “Pale Ochraceous-Buff” when young, becoming “Salmon Color” to “Orange-Pink”, often discoloring “Peach Red” to “Scarlet” near margin of pileus.

Stipe. 36–54 mm long, 2.5–3.0 mm wide at apex, gradually enlarging down-ward or sometimes bulbous to clavate, 3–5 mm wide at the base, “Maize Yellow” to “Buff-Yellow” at apex, “Mustard Yellow” on the lower half with numerous floccose-fibrillose patches; partial veil leaving a loose median annular zone.

Pileus covering. A trichoderm of erect, cylindrical, slightly yellow-brown pig-mented, $117\text{--}188\text{--}345 \times 7\text{--}9\text{--}11 \mu\text{m}$ elements with obtuse to slightly acute apices, commonly secondarily septate, arising from numerous short, cylindrical to clava-te elements near the base, though some elements have an intermediate length (Fig.2).



Figure 1. Fresh basidiocarps of *Lepiota maculans* (collection JMB 05-08-09-18). Scale bar = 1 cm. Photograph © M.G. Wood.

Spores. 8.8-9.5-11.3 × 4.4-4.9-5.4 μm, Q-value 1.86-1.93-2.11, smooth, oblong amygdaliform in profile, fusiform in frontal view, dextrinoid, not metachromatic in cresyl blue (Fig. 2).

Basidia. 22-27-32 × 8-9-11 μm, clavate to subcylindrical, with four sterigmata.

Cheilocystidia. 13-19-29 × 6.0-7.5-11 μm, clavate to subcylindrical, sometimes clavate pedicellate, occasionally arising sympodially (Fig. 2).

Clamp connections. Present in all tissues.

Habitat. Gregarious, terrestrial, in unkempt grass near edge of deciduous forest.

Specimens examined. TENNESSEE: Cosby, GSMNP, 35°45'39"N, 083°12'38"W, elevation 635 m, leg. J.M. Birkebak, 5-Aug-2009, JMB 05-08-09-18, TENN064381.

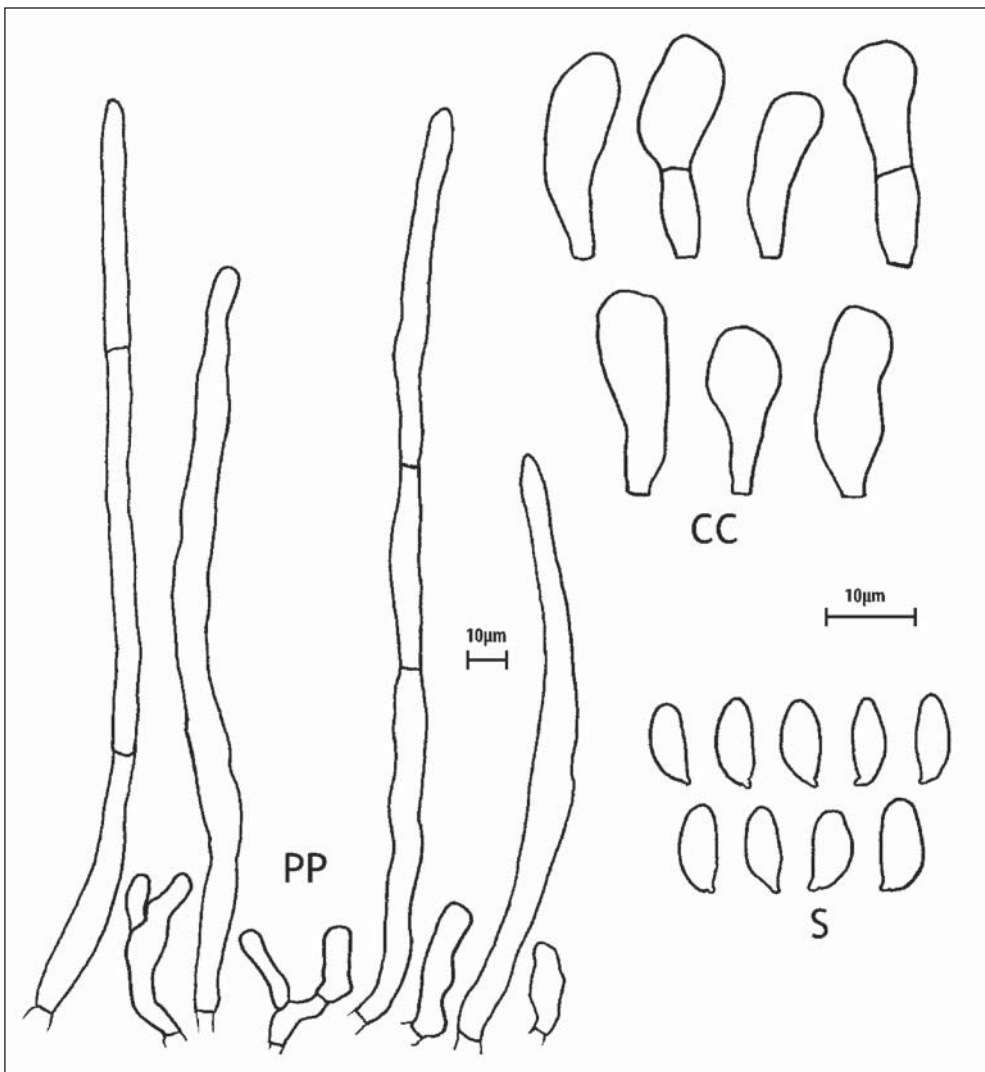


Figure 2. Micromorphological characters: PP = pileus covering, CC = cheilocystidia, S = spores. (from JMB 05-08-09-18).

Comments

This species is set apart in the genus *Lepiota* by its distinctive coloration. Many species of the related genus *Leucoagaricus* discolor orange or red, but no other known members of the genus *Lepiota* discolor so strongly orange and pink. It is unclear whether this coloration change is a staining reaction or a gradual discoloration with age. Peck remarked in his commentary of the protologue that *L. maculans* is easily recognizable due to the bruised flesh that turns reddish and the lamellae that become reddish or pink upon drying. Ideally, future observations of fresh material should resolve whether the nature of discolorations is specifically due to bruising or by maturation of the sporocarps.

Lepiota maculans is the only known species of *Lepiota* to have a "Seashell Pink" spore deposit as opposed to a white or cream deposit for the remainder of the species in the genus. The remaining characters, viz., pileipellis structure, spore morphology, and the presence of clamp connections, are typical for the genus.

Molecular phylogenetic reconstruction using ITS sequence data suggests a placement of *L. maculans* in *Lepiota* sect. *Lepiota* near *L. ignivolvata* Bousset & Joss. described originally from France (Fig. 3). Both species have oblong spores and a pileus covering composed of both long and short elements. *Lepiota ignivolvata*, however, is a robust species with a stipe that discolors orange in its basal part when touched. This species is known only from Europe, where it has been reported widely in numerous floras (Horak 2005).

A study of the holotype (Glatfelter s.n.) at NYS re-affirms our identification of *L. maculans*. Importantly, the spores of the type are consistent in shape, size, and dextrinoid and non-metachromatic walls as the Tennessee material. Unfortunately, due to the poor preservation of the type overall, we could not observe cheilocystidia and trichodermial elements of the pileus covering, although an underlying hymeniderm to the pileipellis was observed (as in our material). As complete a description as possible of the holotype is presented below. The following microscopic observations are our own.

Pileus. 1.5–2 cm diam, thin, convex, subumbonate, dry, minutely and densely squamulose, reddish-yellow, the center darker.

Lamellae. Broad, subdistant, free, white, gradually changing to red or pink.

Stipe. About 5 cm long, 2–3 mm thick, equal, tough, floccose or fibrillose, hollow, whitish or yellowish, the annulus slight, evanescent.

Spores. 7.5–9.5 x 3.7–4.7 μm , ovoid to broadly ellipsoid in profile view, weakly dextrinoid, not metachromatic in cresyl blue.

Basidia. 27–35 x 9–10 μm , clavate, thin-walled, 4-strigmate.

Pleurocystidia. Absent.

Cheilocystidia. Not observed.

Hymenophoral trama. Regular composed by cylindrical to inflated hyphae up to 18 μm broad.

Suhhymenium. Pseudoparenchymatous, up to 20 μm thick.

Pileal trama. Loosely interwoven, composed of cylindrical, radially arranged, thin-walled, 3–12 μm broad hyphae with hyaline or with yellowish red content in KOH.

Pileus covering. Appearing as a hymeniform layer composed of clavate terminal cells, yellowish in KOH.

Stipitipellis. Of parallel and vertically oriented elements up to 15 μm broad, with terminal cells similar to those of the pileipellis and forming tufts more abundant at the apex.

Clamps connections. Present in all tissues.

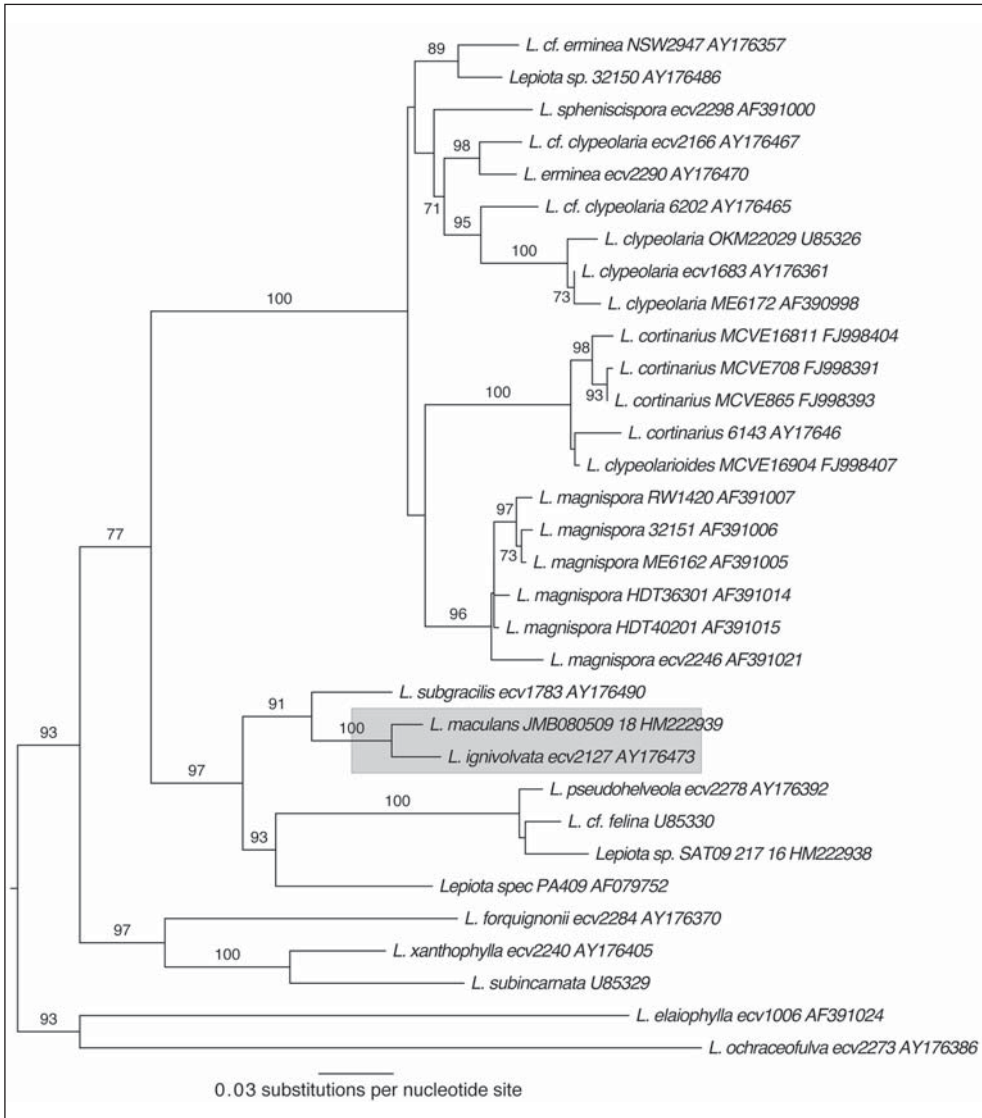


Figure 3. Maximum Likelihood phylogram recovered for the nrITS region using RAxML version 7.2.3 for selected taxa in *Lepiota* sect. *Lepiota* from North America and Europe, including one other species from the GSMNP, with bootstrap values indicated at branch nodes.

Discussion

Basic knowledge of mushroom-forming fungi is relatively poor compared to that of plants and animals. The fruitbodies of mushroom-forming fungi are often ephemeral, and the taxonomy of many species relies on morphological traits necessary to observe in fresh condition. Basic information such as distribution, ecology, and phenology is at best preliminary, if not incomplete, for many fungi of North America, including the southeast United States (Bessette et al. 2007, Weber and Smith 1985).

Extensive survey work documenting fungal biodiversity in the Great Smoky Mountains National Park for the All Taxa Biotic Inventory has been conducted (e.g., Lickey et al. 2007). As of 2009, 833 new records for the Park have been made, 75 of which are species new to science, adding to the 2550 previously reported species to increase the total of known fungi in the Park to 3383 (http://www.dlia.org/atbi/new_science/discoveries.shtml). *Lepiota maculans* represents yet another new record to the Park. It is possible that *L. maculans* has been previously overlooked, but this seems unlikely given its striking coloration and the intensive collecting efforts in the Great Smoky Mountains National Park. It is more likely the species is rare or infrequent given its currently known distribution—Missouri and eastern Tennessee.

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