



## *Smithiomyces dominicanus* (Agaricales: Agaricaceae), a new species from the Dominican Republic

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### Abstract

*Smithiomyces dominicanus* is described as new, based on collections made in the Dominican Republic. Morphologically this taxon is very similar to *S. mexicanus*, but differs in the non-umbonate pileus, the broad stipe base and the smooth basidiospores as seen under light microscope, but with isolated, small warts under SEM. Molecular (nrITS) data also supports the recognition of this taxon as a separate species.

### Introduction

The genus *Smithiomyces* Singer was created to accommodate *Leucomyces mexicanus* Murrill (1911: 80), a tropical lepiotoid species originally described from the state of Veracruz (Mexico), and characterized by the white basidiocarps, velar remnants on pileus and stipe, non-dextrinoid, non-metachromatic and rough basidiospores, globose elements in the pileus covering and abundant clamp-connections (Singer 1944; Horak 1968; Vellinga 1999). The neutral term “pileus covering” is used here for all the covering layers of the pileus, not taking into account their origin (Vellinga 2001a: 109). A second species, *Lepiota lanosofarinosa* Rick (1937: 335), originally described from Brazil, was later included in *Smithiomyces* (Raithelhuber 1988).

Over the past ten years, one of the authors (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* Singer, *Lepiota* (Pers.: Fr.) S.F. Gray, *Leucoagaricus* Locq. ex Singer, *Leucocoprinus* Pat., *Smithiomyces*) and are currently being studied and sequenced.

This paper focuses on the description of a new species in the genus *Smithiomyces*, based on morphological and molecular (nrITS) data.

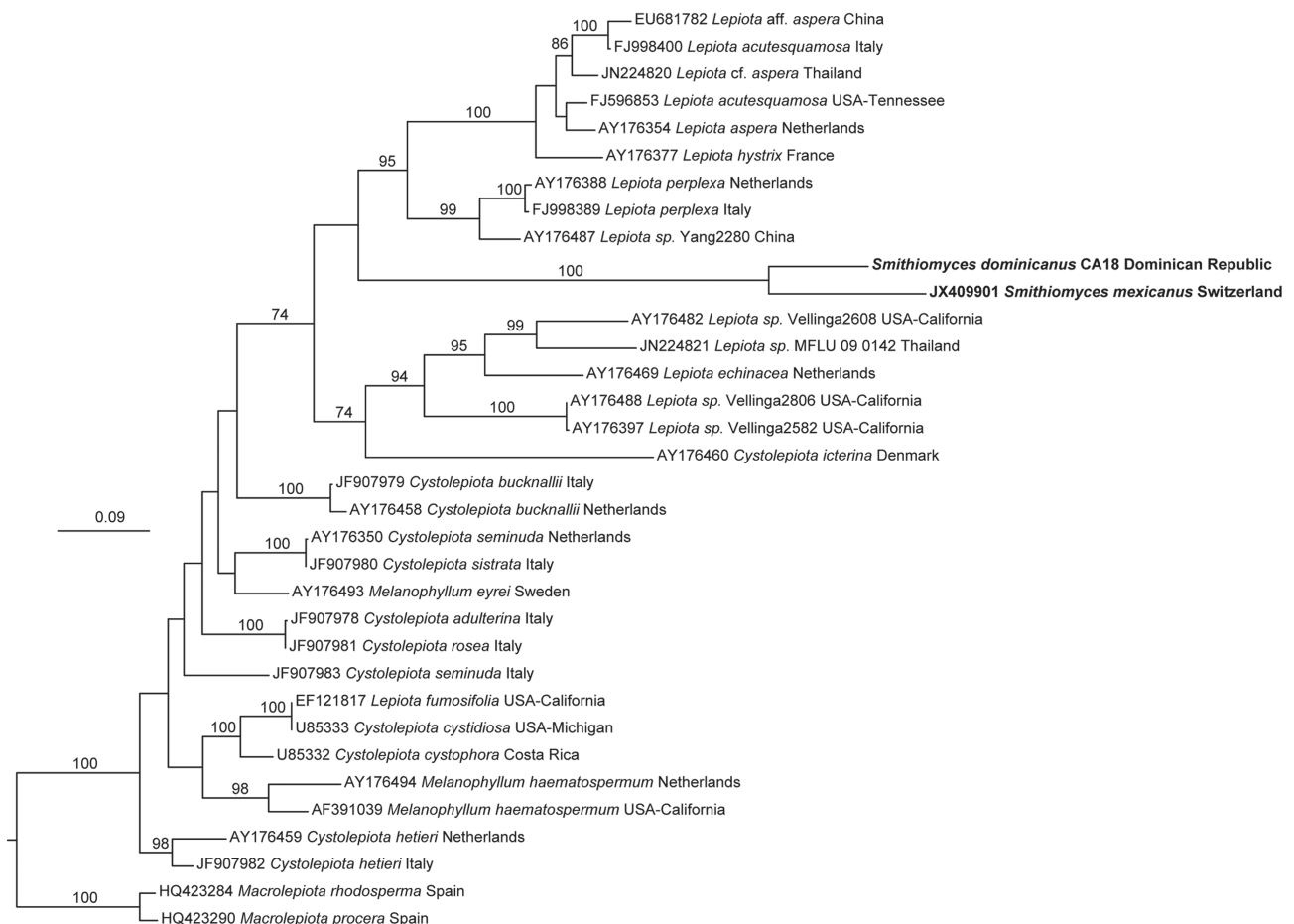
### Material and Methods

**Fungal collections:**—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 & Lanzoni 1990; Vellinga 2001a). Microscopic preparations were mounted in 5% KOH, ammoniacal Congo Red, Melzer’s Reagent and Cresyl Blue. Microscopic observations were made with a Nikon Eclipse E-200 microscope. Descriptive terms for morphological features follow Vellinga (1988, 2001a). The notation [105/5/3] indicates that measurements were made on 105 spores, in five samples, in 3 collections. 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).

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

**Phylogenetic analyses:**—Two different datasets were assembled. A first, preliminary dataset included a total of 357 nrITS sequences, representing the major lineages in the family Agaricaceae. These sequences mainly come from the studies of Baroni *et al.* (2014), Birkebak *et al.* (2011), Caballero *et al.* (2015), Ge & Smith (2013), Kropp *et al.* (2012), Lebel & Vellinga (2013), Liang & Yang (2011a, 2001b), Liang *et al.* (2011), Nawaz *et al.* (2013), Razaq *et al.* (2012), Sysouphanthong *et al.* (2011, 2012), Vellinga (2001a, 2001b, 2001c, 2003, 2004, 2007, 2010), Vellinga *et al.* (2011) and Vizzini *et al.* (2014a, 2014b). Sequences were aligned using MAFFT version 7 (Katoh & 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 one hundred rapid bootstrap (BS) replicates.

After examining the results from this preliminary, we assembled a nrITS dataset with representatives of *Smithiomyces*, *Lepiota* sect. *Echinatae* Fayod, *Melanophyllum*, and *Cystolepiota*, with *Macrolepiota procera* (Scop.: Fr.) Singer (1948: 141) and *Macrolepiota rhodosperma* (P.D. Orton) Migliozi (1995: 140) as outgroup taxa. Sequence alignment and phylogenetic analyses were performed using the same programs and parameters as the preliminary nrITS analysis.



**FIGURE 1.** Best tree from the Maximum Likelihood analysis of the nrITS dataset of *Smithiomyces*, *Cystolepiota*, *Melanophyllum* and *Lepiota* sect. *Echinatae*. Bootstrap values over 70% are shown on or below the branches.

## Results

**Preliminary nrITS dataset of the Agaricaceae:**—The dataset includes 357 nrITS sequences of the major genera in the family Agaricaceae, following the taxon sampling of Vellinga (2003) and Vellinga *et al.* (2011). No outgroup taxa were included, and therefore the midpoint rooting option was selected. The sequence of *S. dominicanus* groups with

*S. mexicanus* with 100% bootstrap support (BS). Both species appear in a more inclusive clade with representatives of *Lepiota* sect. *Echinatae*, *Cystolepiota*, *Coprinus* and *Melanophyllum*, although this grouping receives no support. This tree is not shown here, but it is available together with the alignment files, at TreeBASE (<http://purl.org/phylo/treebase/phylovs/study/TB2:S17615>).

**Reduced nrITS dataset:**—The dataset includes both *Smithiomyces* species, together with 30 nrITS sequences representing taxa in *Lepiota* sect. *Echinatae*, *Cystolepiota*, and *Melanophyllum*. *S. dominicanus* and *S. mexicanus* are grouped together with 100% BS, and both taxa appear in a more inclusive clade with species of *Lepiota* sect. *Echinatae* and *Cystolepiota icterina*, with a BS support of 74% (Fig. 1). In general, the backbone of the tree is not well supported.



**FIGURE 2.** *Smithiomyces dominicanus*. a Basidiocarps (coll. CA18); Basidiocarps (coll. ANGE180); c. Basidiocarps (coll. ANGE181); d. Basidiospores (scale bar=10  $\mu$ m); e. Basidia (scale bar=20  $\mu$ m); f. Cheilocystidia (scale bar=20  $\mu$ m); g Pileus covering (scale bar=100  $\mu$ m). All photographs by Claudio Angelini. All microscopic drawings by Alberto Bizzi and Alfredo Justo (coll. CA18).

## Taxonomic Part

*Smithiomyces dominicanus* Justo, Angelini & Bizzi, *sp. nov.* Figs 2, 3  
Mycobank 812588

Diagnosis:—Differs from *Smithiomyces mexicanus* in the non-umbonate pileus, broad stipe base, smooth basidiospores as seen under light microscope (with isolated small warts under SEM), and nrITS sequence.

Type:—DOMINICAN REPUBLIC. Prov. Puerto Plata: Sosúa, Puerto Chiquito, on humus, 25 November 2011, C. Angelini (CA18-JBSD 126144 holotype; isotype at MEXU). nrITS KR604686 (from holotype).

Etymology:—*dominicanus* refers to the geographic distribution of the species.

Pileus 30–45 mm in diameter, hemispherical or campanulate when young, expanding to convex or plano-convex, without an umbo; surface smooth to innately radially fibrillose, towards the margin with minute, fibrillose-felted to granulose squamules; white, developing yellow or yellow-brown tinges (10YR 8/6, 8/8, 7/6, 7/8) with age or after handling; dry or slightly viscid when moist; margin not striate, appendiculate with abundant, fibrillose remnants of partial veil. Lamellae very crowded, free, segmentiform, up to 1 mm broad; white, developing yellow or yellow-brown tinges with age or after handling; with even or white and flocculose edges. Stipe 30–50 × 5–7 mm, cylindrical, with slightly broad or markedly bulbous base; surface white, smooth or with innate longitudinal fibrils; with a membranous, white ring, usually dissociated in patches in the upper third of the stipe. Context in stipe and pileus, white. Smell and taste farinaceous.

Basidiospores [105/5/3] (4)4.5–6 × 2.5–3(–3.5) μm, avl × avw = 4.9 × 2.7 μm, Q = 1.50–2.40, avQ = 1.81, ellipsoid, oblong or subcylindrical, non-dextrinoid to faintly dextrinoid in Melzer's, non-metachromatic in Cresyl Blue; the spores appear smooth as observed with a light microscope; under SEM the surface appears smooth, slightly rugose or with isolated, small warts (Fig. 3). Basidia 11–20 × 5–9 μm, tetrasterigmate, clavate. Pleurocystidia absent. Lamellar edge sterile. Cheilocystidia 14–29 × 7–14 μm, clavate to narrowly clavate, hyaline, thin-walled. Pileus covering made up of (sub)globose elements, 21–71 × 20–65 μm, hyaline, with slightly thickened wall; intermixed with cylindrical hyphae, 2–5.5 μm wide. Stipe covering with scattered (sub)globose elements, similar to those on pileus. Clamp connections present and common on the pileus covering, also observed at the base of basidia and cheilocystidia, but not at every septum.

**Habit, habitat and phenology:**—Gregarious, in groups of 2 to 5 basidiocarps; growing on the humus layer. In broad-leaved forests, with strong anthropogenic influence. November–December.

**Distribution:**—Only known from two nearby localities in the Dominican Republic.

**Additional collections examined:**—DOMINICAN REPUBLIC. Prov. Puerto Plata: Sosúa, Playa Sosúa, 19 December 2013, C. Angelini ANGE 180 (JBSD); ibidem, ANGE 181 (JBSD).

**Observations:**—*Smithiomyces dominicanus* appears in all phylogenetic analyses (Fig. 1.) as the sister species of *S. mexicanus*, always with 100% support. The nrITS sequences of both species have a percentage similarity of 87%. In morphological terms both taxa are overall very similar, although *S. mexicanus* differs from *S. dominicanus* by having a distinctly umbonate pileus, abundant veil remnants at the stipe base, a non-bulbous stipe base, and rough spores (as seen with a light microscope) that are completely covered in warts (as seen under SEM; see Vellinga 1999). The basidiospores of *S. dominicanus* appear smooth as observed under light microscope, and under SEM the surface of the spores can either be smooth, rough or with isolated warts that do not completely cover the surface (Fig. 3).

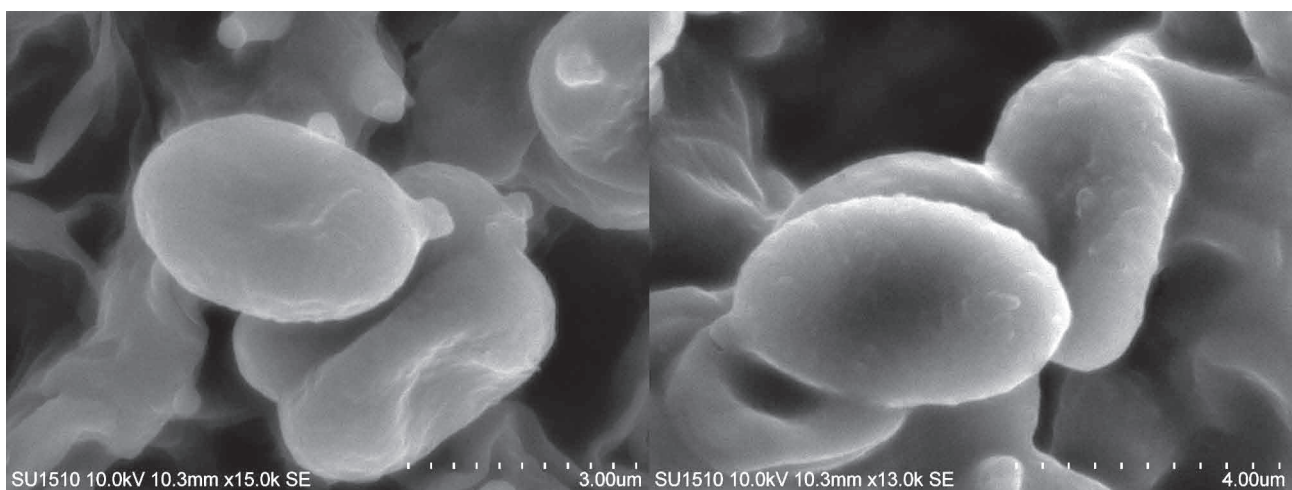


FIGURE 3. *Smithiomyces dominicanus*. Basidiospores as seen under Scanning Electron Microscope (from coll. CA18). Photographs by Berenit Mendoza Garfias.

*Smithiomyces mexicanus* is known from Mexico (Murrill 1911), the state of Florida in the USA (Singer 1944) and Brazil (Horak 1968). It also has been recorded from a Belgian swimming pool, fruiting with imported tropical plants (Vellinga 1999), and from the “Masoala-Hall” in the Zurich Zoo, again with tropical plants (Wilhelm 2015).

There is only one other species in the genus *Smithiomyces*, *S. lanosofarinus*, originally described, and thus far only known, from Brazil (Rick 1939; Raithelhuber 1988). This taxon differs from *S. dominicanus* by having a white pileus with dark red tints, sulphur yellow lamellae and warty spores as seen under light microscope.

Another tropical, overall white species is *Cystolepiota pseudogranulosa* (Berk & Broome) Pegler (1986: 583), which differs from *S. dominicanus* by having a pulverulent pileus surface, smaller basidiocarps (pileus up to 2.5 cm in diameter), cylindrical cheilocystidia, and oblong elements in the pileus covering (Dennis 1952, Pegler 1986; Vellinga 2001a).

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