

***Mayamontana coccolobae* (Basidiomycota), a new sequestrate taxon from Belize**

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Abstract—A new semi-hypogeous, sequestrate genus and species in the *Basidiomycota* is described from the Maya Mountains of Belize, where it was fruiting in association with *Coccoloba belizensis*. *Mayamontana coccolobae* is characterized by small, bright orange basidiomata with a friable, loculate, red-orange to red gleba and bilaterally asymmetric, ellipsoid to subglobose, hyaline to pale green or yellow spores with a slightly wrinkled utricule and thick walls.

Keywords: taxonomy, distribution

Introduction

While collecting basidiomycetes in the Maya Mountains of Belize as part of a US National Science Foundation funded project, one of us (DJL) encountered bright orange, semi-hypogeous, sequestrate basidiomes under *Coccoloba belizensis* Standl. (*Polygonaceae*) and other regionally endemic ectomycorrhizal hosts. The red-orange, loculate gleba bore hyaline, asymmetrical ballistospores with a wrinkled utricule, unlike other genera of sequestrate fungi. A new genus and species, *Mayamontana coccolobae*, is described from these collections.

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Belize is on the southwestern corner of the Yucatan Peninsula, adjacent to México, Guatemala and Honduras and is part of the old terrain of Central America. The upper parts of the Maya Mountains, located in Belize and adjacent parts of Guatemala and Chiapas, México, have remained above sea level as isolated islands during interglacial periods and may have served as refugia for ancestral lineages of various taxa. The basidiomes of the new taxon were primarily associated with the ectomycorrhizal tree, *Coccoloba belizensis* in the Mountain Pine Ridge and Vaca Plateau regions, but also with species of *Neea* (*Nyctaginaceae*) at lower elevations in Blue Hole National Park. The Mountain Pine Ridge near the Five Sisters Falls is granitic, whereas the Vaca Plateau at the Rio Frio Cave Park and the Maya Mountain foothills at Blue Hole National Park are on limestone.

Materials and methods

Methods of collecting and macroscopic and microscopic study were generally those of Castellano et al. (1989). Colors of fresh specimens are in general terms. Hand-cut sections of both fresh and dried material were mounted in water, 5% KOH, Melzer's reagent, or cotton blue for standard light microscopy. Measurements of structures are from mature specimens and, when two dimensions are given, the length precedes the width. Photomicrographs are from material mounted in 5% KOH. Herbaria are abbreviated according to Holmgren et al. (1990). All specimens are deposited in the Mycological Herbarium of Oregon State University (OSC) and also in Forest Department Herbarium, Belize (BRH).

DNA was extracted from basidiome tissue using the CTAB/chloroform method described by Gardes & Bruns (1993), except that the source tissue was not lyophilized. The DNA extract was purified with the Q-Biogene GeneClean II glassmilk kit, and the ITS region of the nrDNA was amplified with primers ITS-1F and ITS-4 using the following PCR protocol: 1) 5 m at 95 C; 2) 1 m at 95 C; 3) 1 m at 52 C; 4) 2 m at 72 C; 5) repeat from step two 34 times. Success of the amplification was checked by electrophoresis on a 2.5% agarose gel.

Successfully amplified PCR product was again cleaned using the Q-Biogene GeneClean II glassmilk kit. Concentration of DNA in the PCR product was estimated by electrophoresing the product on a 2.5% agarose gel with a calibrated marker in an adjacent lane. The purified PCR product was diluted to an approximate molecular weight of 50 ng in 7 μ l of dH₂O. To this dilution was added 0.6 μ l of primer ITS-4, 1 μ l of sequencing buffer, and 2 μ l of ABI Prism Big Dye Terminator 3.1. This cocktail was subjected to the following PCR protocol: 1) 5 m at 96 C; 2) 30 s at 96 C; 3) 15 s at 50 C; 4) 4 m at 60 C; 5) repeat from step two 25 times.

Sequencing was performed by the Oregon State University Center for Genome Research and Biocomputing on an Applied Biosystems capillary 3730 Genetic Analyzer. The sequences were submitted to the BLAST search engine for matching at the National Center of Biotechnology Information.

The large subunit of nrDNA of one specimen was also amplified and sequenced. Methods were as above except primers LR0R and LR3 were used for initial PCR amplification and LR0R was used for sequencing. BLAST results (below) were consistent with those for the ITS region.

Taxonomic description

Mayamontana Castellano, Trappe & Lodge, gen. nov.

MYCOBANK MB510440

A Stephanosporaceis altris gleba pallide rubroaurantia vel rubra et sporis bilateraliter asymmetricis, laevibus, utriculo rugoso, pariete crasso, singulatim hyalinis vel pallide viridibus vel luteis, in massa pallide viridiluteis, pedicellatis.

Etymology: *Mayamontana* honors the Mayan people and refers to the Maya Mountains.

Type species: *Mayamontana coccolobae* sp. nov.

Mayamontana coccolobae Castellano, Trappe & Lodge, sp. nov.

Fig. 1

MYCOBANK MB510441

Basidiomata 6-8 mm lata, vivide aurantia. Gleba loculata, friabilis, pallide rubroaurantia vel rubra. Sporae (-9) 10-12 (-14) x (5-) 6.5-7 (-8) μm, bilateraliter asymmetricae, laeves, utriculo parum rugoso, pariete plus minusve 2 μm crasso, singulatim hyalinae vel pallide virides vel luteae, in massa pallide viridilutea, pedicello 3 x 2 μm. Holotypus BZ-1943.

Etymology: *coccolobae* refers to the association of this species with the plant, *Coccoloba belizensis*.

Basidiomata beneath loose litter or exposed, attached to the surface of mineral soil, attachment point evident at base where the peridium does not enclose the gleba, when fresh 6-8 mm in diameter, ovoid, bright orange, slightly felty, as dried 2-3 x 2-5 mm, irregularly flattened, subglobose to globose, pale orange-tan to orange to bright orange, slightly felty, very wrinkled; **gleba** when dried friable, pale red-orange to red, when rehydrated in 5% KOH the mediostratum of the trama is often bright red; spores and hyphae partially filling the irregularly rounded locules, 0.1-0.2 mm broad, red-orange leachate apparent when mounted in 5% KOH; **columella** lacking; **odor** none; **taste** not recorded.

Peridium 100-125 μm thick, of pale orange, thin-walled, inflated cells and irregularly interwoven to subparallel hyphae (5-10 μm in diam); the tissue near the outer surface encrusted with debris; the tissue near the gleba tends

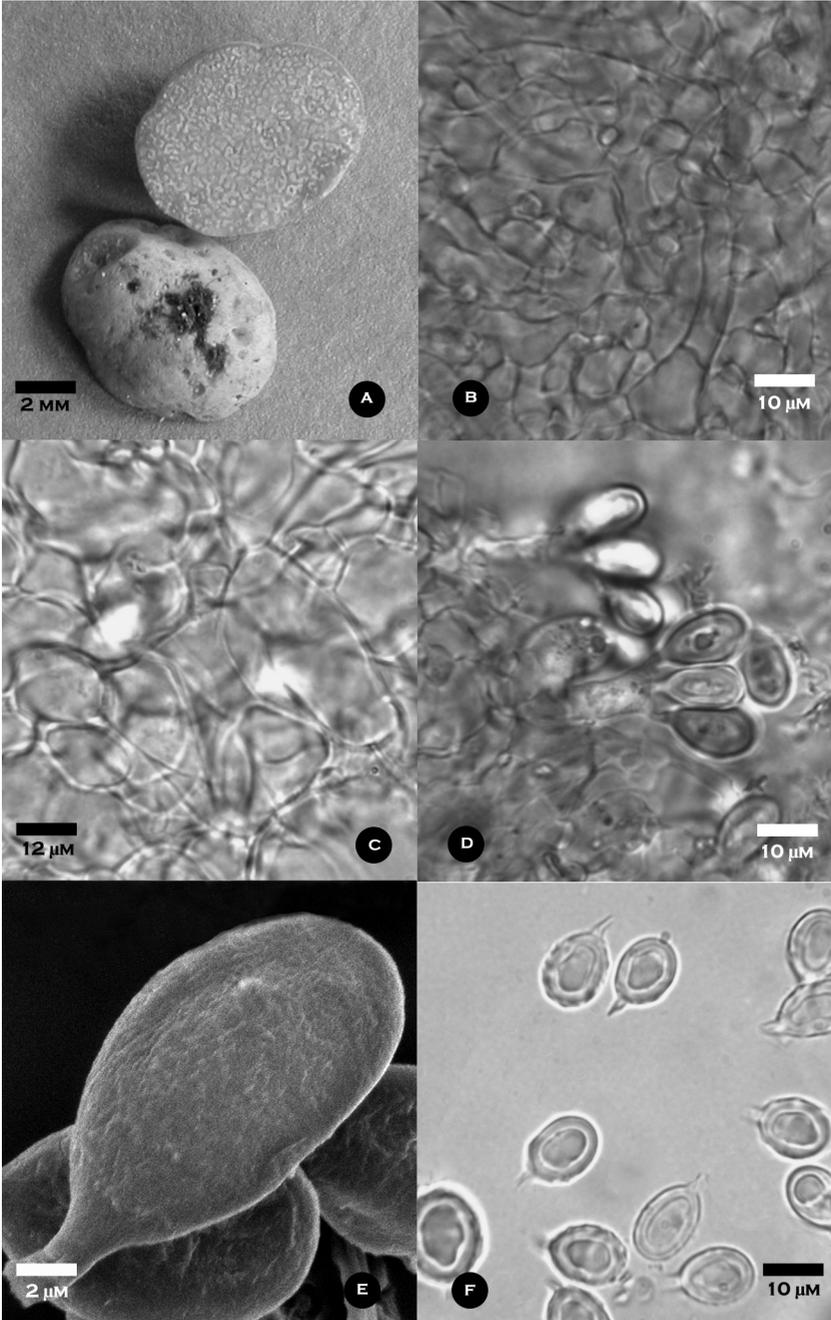
to have more interwoven hyphae than inflated cells, but no clear demarcation of peridial layer boundaries; **trama** similar in structure to the peridium but more pale to nearly hyaline, of thin-walled cells irregularly inflated, up to 17 x 39 μm , mostly about 15 x 20 μm ; **subhymenium** of hyaline, thin-walled, cells irregularly inflated up to 5mm diam; **hymenium** a palisade of basidia and basidioles, collapsing soon after spore development and obscured by the mass of spores; **cystidia** absent; **basidia** hyaline, thin-walled, subclavate, 25-37 x 7-10 μm , 4-spored; **sterigmata** 2-6 μm tall, 1-2 μm wide at base, hyaline; **clamp connections** absent; **spores** (9-) 10-12 (-14) x (5-) 6.5-7 (-8) μm , asymmetrical, broadly ellipsoid to subglobose, slightly flattened on one side, smooth; utricle uneven, slightly wrinkled, not inflated, giving the spore a roughened to warty appearance, thick walled, up to ± 2 μm thick; inner surface of the spore wall appearing uneven, in KOH mostly hyaline but sometimes slightly pale green or yellow singly, in mass pale green-yellow, in Melzer's reagent no reaction; spore walls 0.5 μm thick; **pedicel** distinct, up to 3 μm long and 2 μm wide; the end appearing ragged.

Ecology, range, and distribution—Seimi-hypogeous, scattered, on soil under loose leaf litter, beneath *Coccoloba belizensis* and *Neea* sp. at 70-370 m elevation in the Maya Mountains of Belize; August and October.

REPRESENTATIVE SPECIMENS EXAMINED—BELIZE: MAYA MOUNTAINS, MOUNTAIN PINE RIDGE, FOREST RESERVE, FIVE SISTERS LODGE, LOWER NATURE TRAIL (88° 59' 8" W 17° 2' 16"N), D.J. LODGE, BZ-3052, 5.X.2003 (HOLOTYPE-BRH; ISOTYPE-OSC). BELIZE: SAME LOCALITY AS HOLOTYPE EXCEPT, UPPER NATURE TRAIL (88° 59' 8" 17° 2' 19.4"N), D.J. LODGE, BZ-74, 10.VIII.2001 (BRH; OSC); BLUE HOLE NATIONAL PARK, MAYA MOUNTAINS, NORTHERN FOOTHILLS, CAVES BRANCH, ALONG HUMMINGBIRD LOOP TRAIL (88° 41' 1.5"W 17° 9' 29.5"N), D.J. LODGE, BZ-1943, 30.X.2002 (BRH; OSC).

Comments—*Mayamontana* is characterized by the small, hyaline, asymmetrical, smooth spores with a roughened utricle and a distinct pedicel, unusual in sequestrate taxa. The spores are similar to those of *Sclerogaster* in overall appearance but are significantly larger in size and lack the distinct warts of *Sclerogaster* spores. Basidiome characters are reminiscent of *Stephanospora* in peridial color, size, texture of the peridium and fruiting habit. We extracted DNA material from *M. coccolobae* and upon performing a BLAST search using GENCOM it is placed in the *Stephanosporaceae* (Martin et al. 2004) near *Stephanospora caroticolor* (Berk.) Pat. (GenBank Accession AJ419224). The characteristics of the basidiome are somewhat similar to species of

Fig. 1. *Mayamontana coccolobae* (holotype). A. Basidioma, B. Peridial hyphae in radial section; C. Tramal cells in radial section; D. Basidia and spores attached to sterigmata; E. SEM of basidiospore with distinct pedicel; F. Light photomicrograph of basidiospores showing the ragged end of the pedicel.



Stephanospora but basidiomes of *Stephanospora* species have a yellow peridium and a gray-olive gleba. *Mayamontana* also differs markedly from all described *Stephanospora* taxa by the lack of a basal ornamental collar or “corona” surrounding the spore base and prominent, wedge-shaped ridges.

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