Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
10(1): 96–102 (2020) ISSN 2229-2225
www.creamjournal.org

Article

Doi 10.5943/cream/10/1/10

Agaricus pocillator, a new record species from Mexico

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Chen J, Enríquez-Bedolla JC, Rugolo, Llarena-Hernández RC 2020 – *Agaricus pocillator*, a new record species from Mexico. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 10(1), 96–102, Doi 10.5943/cream/10/1/10

Abstract

Agaricus is a species-rich genus with nutritional and medicinal interest. Though the taxonomy and phylogeny of the genus have been well advanced during the last decade, the species diversity of the genus has been little studied in Mexico. In the present study, we introduce *A. pocillator* as a new record species from Mexico based on both morphological characters and molecular data. The full description, line drawings and photos are provided.

Key words – Agaricaceae – taxonomy – ITS

Introduction

The genus *Agaricus* L. comprises saprophytic mushrooms, which are characterized by sporocarps with free lamellae, an annulate stipe and a dark brown spore print. During the last decade, the taxonomy and evolutionary phylogeny of the genus have been well advanced, with more than 500 species recognized worldwide (Callac & Chen 2018, Chen et al. 2019, Zhao et al. 2016). Numerous *Agaricus* species are appreciated for their delicate taste as well as their nutritional and medicinal values, such as *A. bisporus* (J.E. Lange) Imbach, the most cultivated species in the world (Mata et al. 2016). Members of *A.* sect. *Xanthodermatei* may be potentially toxic, therefore, none of the species of this section is recommended for consumption (Parra 2013).

The *Agaricus* diversity in Mexico is not well studied. The latest review focusing on tropical and subtropical species of *Agaricus* in Mexico was conducted by Medel et al. (2018). In total, 57 *Agaricus* species were recorded in Mexico from temperate, tropical and subtropical forests (Medel et al. 2018, Chen et al. 2019). However, due to the lack of molecular data, some of the identifications may remain doubtful.

Agaricus pocillator Murrill belongs to *A.* sect. *Xanthodermatei*, which is a well-known species in the southeastern United State characterized by its cup-like bulb stipe (Kerrigan 2016). During a survey of wild mushroom in Veracruz State, Mexico, numerous *Agaricus* species were collected. The present study aims to describe *A. pocillator* as a new record species from Mexico based on both morphological characters and molecular data.

Materials & Methods

Submitted 20 February 2020, Accepted 2 March 2020, Published 6 March 2020
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Sampling and morphological study

The specimens studied were collected in Veracruz State, Mexico, in 2019. Specimens are deposited in the Institute of Ecology INECOL (XAL Herbarium). Specimens were photographed *in situ* and in laboratory. Odor and color change (when rubbed or cut) were recorded in laboratory. The macroscopic characters were recorded following the methodology described by Largent (1986). KOH and Schäffer's reactions were performed as described by Chen et al. (2015). Micromorphological features were examined from dried specimens following the protocols of Largent et al. (1977) including anatomy of basidiospores, basidia, cystidia, pileipellis and partial veil. Measurements of basidiospores were presented based on at least 20 measurements and include x = the mean of length by width; Q = the quotient of basidiospore length to width, and Q_m = the mean of Q-values.

DNA extraction, PCR and sequencing

DNA was extracted from dried specimens using a commercial DNA extraction kit (Plant/Fungi DNA Isolation Kit, Norgen Biotek Corp.). The internal transcribed spacer (ITS) region was amplified following the protocol of Zhao et al. (2011), by using primers ITS4 and ITS5. PCR products were purified and sequenced at Macrogen Inc., Korea.

Phylogenetic analyses

The dataset comprises five sequences from specimens collected in the present study, in addition to 61 sequences retrieved from GenBank (Zhou et al. 2016, Parra et al. 2018, Phookamsak et al. 2019, Kerrigan 2016, Zheng et al. 2019). GenBank accession numbers are indicated in the phylogenetic tree (Fig. 1). Sequences were aligned in MAFFT (Katoh & Standley 2013), then manually adjusted in BioEdit v. 7.0.4 (Hall 2007). The alignment has been submitted to TreeBase (submission ID 25833). The dataset was partitioned into ITS1, 5.8S and ITS2 regions. The maximum likelihood (ML) analysis was performed in RAxMLHPC2 v. 8.2.4 (Stamatakis 2014) as implemented on the Cipres portal (Miller et al. 2010), under a GTRGAMMA model with one thousand rapid bootstrap (BS) replicates. Bayesian Inference (BI) analysis was performed with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Six Markov chains were run for one million generations and sampled every 100th generation. Burn-in was determined by checking the likelihood trace plots in Tracer v. 1.6 (Rambaut et al. 2014) and subsequently discarded. The outputs were displayed in FigTree v 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Phylogenetic analyses

The dataset comprises 66 ITS sequences representing 59 *Agaricus* species of three sections. The final alignment contains 697 characters including gaps. *Agaricus campestris* L. was used as outgroup. The resulting ML and Bayesian trees had highly similar topologies. The ML tree is shown in the Fig. 1 and branches with Bayesian posterior probabilities higher than 90% are thicker. According to the ITS tree, *A*. sect. *Xanthodermatei* is monophyletic, but lacks significant statistical support, which agrees with previous studies (Parra et al. 2018, Zhou et al. 2016). The five Mexican samples clustered with three samples of *A. pocillator* downloaded from GenBank with strong support. Phylogenetically, *A. pocillator* is sister to *A. tollocanensis* Callac & G. Mata, a species only known from Mexico. And the two sister species form a strongly supported clade with *A. candussoi* L.A. Parra, Angelini & Callac.

Taxonomy

Agaricus pocillator Murrill, Mycologia 33(4): 446 (1941) Figs 2, 3

Macroscopic description – Pileus 45–75 mm diam., hemispherical to truncate-convex when young, then plano-convex, finally plane at maturity; surface dull and dry, covered with greyish brown fibrils over a beige background, densely at disc and form an entire dark brown center; or sometimes with an entire white cap, and with light brownish or ochre tinges at disc. Margin not exceeding the lamellae. Lamellae free, straight, intercalated with numerous lamellulae, white, becoming pink, finally brown at maturity. Stipe 8.5–10 × 0.5–1 cm, cylindrical or slightly tapering at base, and with a cup-like bulbous base about 0.7–1.4 cm broad, whitish, becoming yellowish when bruised, smooth. Annulus apical, double, white, thin at the insertion but thicker at the margin, upper surface smooth, lower surface cottony, covered with irregular scales. Sometimes the thicker part of the margin is divided into two parts, and the inferior part forms a bracelet near to the stipe. Context white, firm, no discoloration when cut, or slightly yellowing at the basal part of the stipe. Odor of medium to strong phenol.

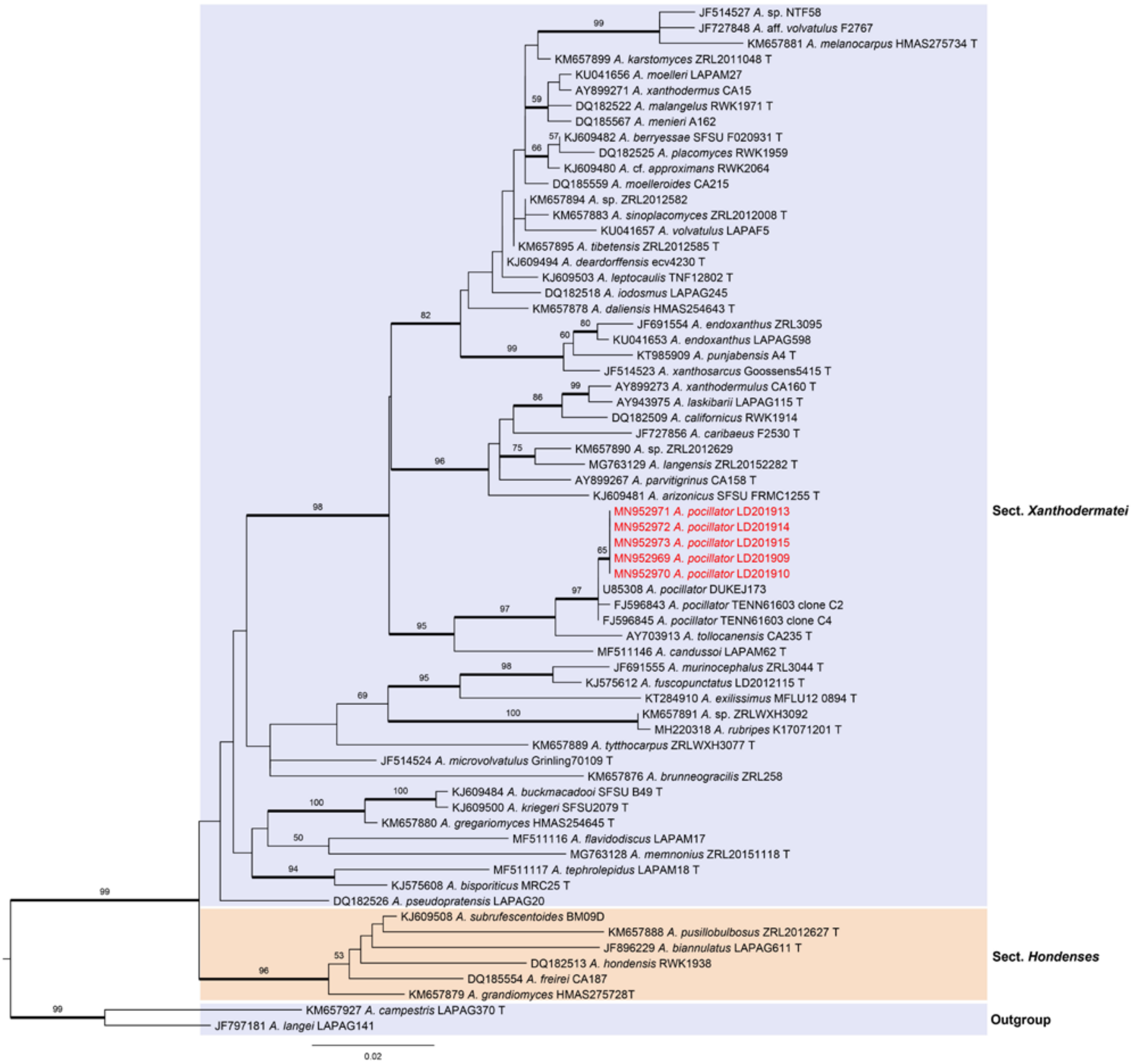

Fig. 1 – Maximum likelihood phylogram of *Agaricus* sect. *Xanthodermatei* resulting from analysis of ITS sequence data. The bootstrap support values greater than 50% are indicated, and branches with Bayesian posterior probabilities greater than 0.9 are thicker. Mexican samples of *A. pocillator* are in red. T = Type specimen.

Microscopic description – Spores (4.4–)4.6–5.8(–6) × 2.8–3.5 µm, (x = 5.1 × 3.2 µm, Q = 1.37–1.86, Q_m = 1.59, n = 40), ellipsoid, smooth, brown, without apical pore. In colored sporocarps, alongside these mature dark brown spores there are also abundant hyaline spores among which are frequently giant spores of 6.4–7.1 × 3.1–3.8 µm, Q=1.67–2.12, ellipsoid-elongated. Basidia 12–16.5 × 5–6 µm, bisporic to tetrasporic, clavate to broadly clavate, hyaline, smooth. Cheilocystidia abundant, hyaline, simple, cylindrical to sub-clavate, 10–30 × 5–7 µm. Pleurocystidia not observed. Lower surface of annulus composed of cylindrical hyphae, not or slightly narrowed at the septa, 3–7 µm wide. Inflated elements not observed. Pileipellis a cutis composed of cylindrical and thin-walled hyphae of 2.5–10 µm wide, hyaline or with brownish diffuse or vacuolar pigments; the terminal elements can be cylindrical or progressively attenuated with rounded apex or clavate of 10–19 µm wide. Clamp connections not observed.

Macrochemical reactions – Schäffer's reaction negative. KOH reaction positive, yellow.

Habitat – scattered in soil covered with leaves litter, in Mesophyll forest.

Material examined – Mexico, Coatepec, Cascada La Granada, site 1, 16 June 2019, M. Rugolo, LD201909; Coatepec, Cascada La Granada, site 1, 16 June 2019, M. Rugolo, LD201910; Coatepec, Cascada La Granada, site 2, 16 June 2019, M. Rugolo, LD201913; Coatepec, Cascada La Granada, site 2, 16 June 2019, M. Rugolo, LD201914; Coatepec, Cascada La Granada, site 2, 16 June 2019, M. Rugolo, LD201915.

Fig. 2 – Macroscopic characteristics of *Agaricus pocillator*. a–d Sporocarps. a (LD201910). b (LD201909). c–d (LD201913). Scare bars = 10 mm.

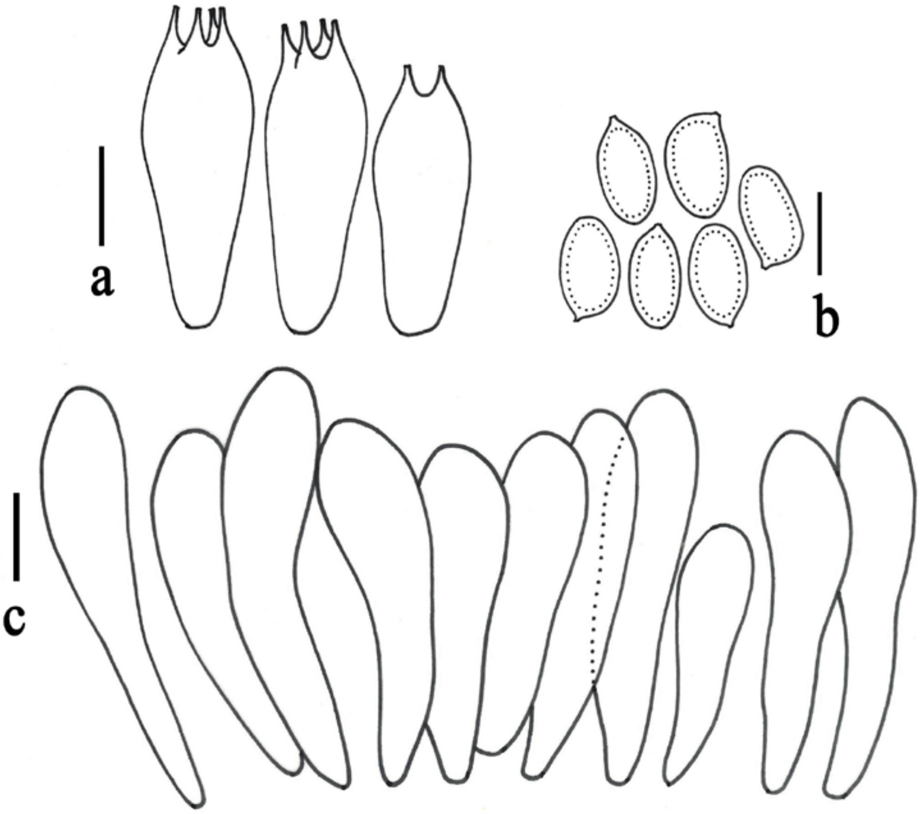

Fig. 3 – Microscopic characteristics of *Agaricus pocillator*. a Basidia. b Basidiospores. c Cheilocystidia. Scale bars = 5 µm.

Notes – *Agaricus pocillator* is remarkable in the field by its cup-like bulbous base and the greyish pileus. It is a regularly encountered species of mixed forests in the southeastern United States (Kerrigan 2016). Our Mexican materials match well with the original description, except the pileus color can be varied from greyish brown to white due to the lack of colored fibrils. Meanwhile, some hyaline macrospores were observed in our collections. Phylogenetically, *A. pocillator* is most related to *A. tollocanensis*, however, the latter has more robust sporocarps, a bulbous stipe but never cup-like, and slightly bigger spores of 5.7 × 4.1 µm in average (Callac & Mata 2004). In addition, their ITS sequences differ at nine positions.

Morphologically, *A. pocillator* resembles other grey woodland *Agaricus* of *A.* sect. *Xanthodermatei*, such as *A. deardorffensis* Kerrigan, *A. leptocaulis* Kerrigan, *A. placomyces* Peck and *A. sinoplacomyces* P. Callac & R.L. Zhao, but none of them forms a marginate bulb (Kerrigan 2016, Zhou et al. 2016). The white sporocarps of *A. pocillator* is quite similar to *A. candussoi* recently described from Dominican Republic, but the latter species differs by its slightly bulbous base, slightly smaller spores of 4.63 × 3.07 µm in average, and different shapes of cheilocystidia (Parra et al. 2018). *Agaricus microvolvatulus* Heinem. and *A. volvatulus* Heinem. & Gooss.-Font. both feature a typically marginate base, greyish pileus surface, and similar sized spores. Despite *A. microvolvatulus* may form sporocarp with pileus diameter larger than 10 cm (Thongklang et al. 2014), and the absence or rarely existence of cheilocystidia in *A. volvatulus* (Chen et al. 2016), ITS sequences remain essential for the unambiguous identification of those species.

Discussion

Five specimens collected in Veracruz, Mexico were identified to *A. pocillator*, and represent a new record species to the country. Our data extend the known distribution range of *A. pocillator* from southeastern United States to central Mexico. Meanwhile, our collections include both greyish fibrillose sporocarps and white smooth sporocarps, which has not been reported in this species before.

In the genus *Agaricus*, the pileus color may vary from entirely white to dark colored with all possible intermediates. In many species, it can vary depending on both environmental and genetic factors, and their interaction (Callac et al. 2005, Parra 2008, Kerrigan 2016). For example, in *A. bisporus*, a major genetic determinant, with some modifier genes are responsible for colors ranging from pure white to dark mahogany brown (Callac et al. 1998, Kerrigan 2016). The correlation between the pileus color and the habitat observed in a previous study suggested that white pileus would represent an advantage in open habitat, possibly by conferring a resistance against dryness due to sunshine (Callac et al. 2005). However, this hypothesis does not apply to *A. pocillator*, which is a woodland species. In our case, two collecting sites were in the forest and about 300 meters far from each other. Our observations suggest there is a genetic variability for the pileus color in this local population of *A. pocillator*.

Acknowledgements

The authors are grateful to Teresa Gutiérrez Leucona for the field trip. Maximiliano Rugolo thanks the Mexican Agency for International Development Cooperation for the research grant. This research was financially supported by the project PRODEP, apoyo a la incorporación de nuevos PTC (DSA/103.5/16/10438).

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