CORTINARIUS WATSONEAE, A NEW SPECIES OF AGARICOMYCETES (CORTINARIACEAE) FROM THE GULF STATES

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ABSTRACT

Cortinarius watsoneae, a new species in subgenus Myxacium, sect. Myxacium, is described from pine and mixed pine and hardwood forests from the Gulf States region of North America. It is characterized by the young lamellae that are grayish violet to pale violet, and relatively large basidiospores in comparison to C. mucosus. The ITS sequence is distinct from other members of sect. Myxacium, with 97% similarity to the closest known species, C. collinitus and C. mucosus. The new species is named in honor of the late Geraldine Watson.

KEY WORDS: new species, mushrooms, biodiversity

INTRODUCTION

Progress is being made in understanding the species-rich diversity of mushrooms that occur in the Gulf States region of the United States. For dark-spored, ectotrophic genera such as Cortinarius and other genera in the Cortinariaceae, the diversity remains poorly documented in comparison to the more northerly temperate and boreal forests of the United States and Canada. It is not the objective here to summarize the history of work done on the Cortinariaceae in the Gulf States region; however, in recent years, new species have been described or reported (e.g., Lewis & Ovrebo 2009; Liimatainen & Niskanen 2021; Niskanen et al. 2013). In addition, the relationship and distribution of previously known species and newly described ones, has been

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enhanced by combining genomic data with morphological and ecological information for North American taxa (e.g., Niskanen et al. 2012; Harrower et al. 2015; Dima et al. 2021). Through this approach 10 genera are now recognized in the Cortinariaceae (Liimatainen et al. 2022). In this paper we present the combined efforts of field work, morphological, and molecular analyses to describe a new species of Cortinarius, C. watsoneae. It belongs in subgenus Myxacium, sect. Myxacium, and is related to C. mucosus (Bull.) Kickx and C. collinitus (Sowerby) Gray.

MATERIALS AND METHODS

Notes and photos were made from fresh material. Color notations were matched with Kornerup and Wanscher (1961). Pileipellis, lamellae and basidiospores were studied from sections and pieces of dried exsiccate. Microscopic examination of basidiospores, pileus and lamella tissues were made in 3% KOH. Q indicates the length/width ratio.

For DNA extraction, fungal tissue was placed in a microcentrifuge tube with 400 µl of Chelex buffer (100 mM Tris pH = 8.5, 4% Chelex 100 (Bio-Rad Laboratories), 1% Triton X-100). The tubes were heated to 99°C for 20 min, then frozen. After thawing, the tubes were centrifuged, and the supernatant was used in PCR. For the Florida sample (14-057) DNA was amplified directly from dried lamellae using the Phire Plant Direct PCR kit (Thermo Fisher Scientific) following the manufacturer's instructions (Liimatainen & Ainsworth 2018). PCR was performed in 25 ml reactions with 1 µl DNA extract, 0.4 mM each primer, 0.2mM dNTP mixture, 5 µg bovine serum albumin, and 0.5 U OneTaq Hot Start DNA polymerase (New England Biolabs) in 1X OneTaq standard buffer. PCR conditions were: 94°C for 30 s, followed by 36 cycles of 94°C for 15 s, 57°C for 30 s and 68°C for 60 s, followed by a final extension at 68°C for 5 min. Primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the ITS1-5.8S-ITS2 region. Sequencing was performed at Eurofins Genomics (Louisville, KY) using the same primers used for PCR. Forward and reverse reads were aligned using Genious 10.2.4 and resolved manually, as needed.

RESULTS

Molecular Analysis

We generated four ITS sequences and included 14 published ITS sequences from GenBank in our dataset (Table 1). A sequence from the genus Calonarius was selected as an outgroup based on Liimatainen et al. (2022). A total of 18 ITS sequences were aligned using MAFFT 7 (Katoh & Standley 2013). The alignment is 673 nucleotides long (including gaps). A phylogenetic tree was generated from the dataset using maximum likelihood (ML) analyses with 1000 bootstrap replicates under the GTRGAMMA model in RAxML 8 (Stamatakis 2014) (Fig. 1).

Taxonomy

Cortinarius, subgenus Myxacium

Subgenus Myxacium contains five sections: Myxacium, Cuphromorphi, Defibulati, Marmorati, and Quadrispora (Soop et al. 2021), two of which, Myxacium and Defibulati, are prevalent in North America. Agaricoid species of these two sections are similar in appearance, with a conic to broadly convex, viscid to glutinous pileus, glutinous to dry cylindrical stipe, and relatively large amygdaloid to citriform basidiospores. Species in sect. Myxacium commonly have clamp connections associated with their hyphae, while clamp connections are essentially absent in sect. Defibulati. Cortinarius watsoneae is a member of sect. Myxacium.

Cortinarius watsoneae Lewis, Ammirati, Liimat., Niskanen, Ovrebo, Justice, & Kaminsky, sp. nov. (Figs. 1, 2). Type: UNITED STATES. TEXAS. Tyler Co.: Hyatt Lake, Watson Rare Native Plant Preserve, 30.5814, −94.3789, 14 Nov 2020, David P. Lewis 13692 (holotype: WTU; isotype: TAES; Mycobank no. MB847491; GenBank no. OQ343665).

Diagnosis.—Characterized by the brown viscid pilei, violaceous colors to the young lamellae and stipe, and large spores, 14.5–17 × 6–8.2 µm. The ITS sequence (GenBank OQ343665, ex holotype) is distinct from other members of C. subgen. Myxacium, sect. Myxacium and with 97% similarity to the closest known species, C. collinitus and C. mucosus.
Etymology.—Named in memory of Geraldine Ellis Watson, renowned Texas naturalist, conservationist, artist, author and ecologist (Clark 2021).

Pileus 30–75 mm broad, convex becoming plano-convex to plane, sometimes with a slight umbo, disc becoming depressed, viscid to dry, glabrous, colored yellowish brown, golden brown (5D7), reddish golden brown to light brown (6C-D7) or dark brown (7F8), becoming more cinnamon brown (6D6) overall in age; context 5–10 mm thick, colored whitish with a cream tint to dingy whitish to brownish orange (5C3); odor and taste mild.

Lamellae adnate to adnexed, ± close, 4–9 mm broad, acute, entire to slightly eroded, at first pale grayish violet, pale violet to purplish and dull brown, becoming rusty brown; lamellulae in 1–2 tiers.

Stipe 20–40 mm long, 7–15 mm thick, ± equal, ± viscid, fibrillose, purplish when young, whitish to dingy whitish when elongated, often tinged violet, yellowish brown on base, context solid, ochraceous buff, with some violet to purple or brownish colors beneath surface, often brown in base; cortina white to pale violet, leaving a conspicuous fibrillar zone mid-stipe.

Chemical color reaction.—Application of 5% KOH on pileus surface dark brown.

Basidiospores 14.5–17 × 6–8.2 µm, (n=30, mean=15.7 × 6.9 µm, Q=2.11–2.49, mean Q=2.29); narrowly amygdaloid, less commonly amygdaloid or somewhat ellipsoid to fusoid, distal end ± extended, distinctly and coarsely verrucose. Basidia 4-spored. Pileipellis: epicutis a ± well developed gelatinous layer of narrow, cylindrical colorless or sometimes yellowish hyphae; hypocutis a distinctly pigmented layer, hyphae interwoven, cylindrical to ± enlarged, but not forming a distinct cellular layer, walls yellow, yellow brown or orange yellow brown, some encrusted, some filled with yellow brown pigment. Lamellae edges with clavate to somewhat cylindrical sterile cells. Clamp connections present and common.

Distribution.—Known from Texas, Mississippi and Florida, widespread and likely misidentified as C. mucosus in herbaria.

Habitat.—Gregarious, often very common, in pine and mixed pine and hardwood upland forests, November and December.
Fig. 1. A phylogram resulting from the RAxML analysis of the dataset. Bootstrap values greater than 50% are indicated above branches.

Fig. 2. Cortinarius watsoneae, basidiomata and basidiospores. A. D.P. Lewis 14164, scale bar=25 mm. B. D.P. Lewis 13692, scale bar=25 mm. C. D.P. Lewis 13692, scale bar=20 μm. D. Geraldine Watson.

Comments.—Cortinarius watsoneae is recognized by the brown, viscid pileus, violaceous colors of the young lamellae and stipe, and by the relatively large basidiospores (Fig. 2). Cortinarius watsoneae is similar in appearance to C. mucosus (Brandrud et al. 1992) but differs by the violaceous colors of the lamellae and stipe, and by the larger basidiospores (14.5–17 × 6–8.2 µm for C. watsoneae and 11.5–14 × 5.5–7 (7.5) µm for C. mucosus). Although Cortinarius watsoneae occurs in pine and mixed pine-hardwood forests, we suspect that it is a pine-associate, and C. mucosus occurs with two-needled pines. Cortinarius collinitus (under C. muscigenus Peck, Brandrud et al. 1990) occurs in conifer forests, has grayish white lamellae when young, and basidiospores 13–16 × 7.5–9.5 µm in size. Both C. mucosus and C. collinitus have a more northerly distribution, while records of C. watsoneae are from the deep south, although the full extent of its distribution will require further study. Other morphologically similar species include. C. maae Ammirati, Halling, Liimat., & Niskanen and C. hallingii Ammirati, Niskanen, Liimat., & Garnica, but both species are associated with species of Quercus in Central America. It may be that the photo in Weber and Smith (1985) that is labelled as C. mucosus is actually C. watsoneae; however, the voucher collection for that photo has not been studied.

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