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.

RESUMEN

Se describe **Cortinarius watsoneae,** especie nueva del subgénero *Myxacium*, sección *Myxacium*, encontrada en bosques mixtos de pino en los estados del Golfo de Norte América. Las setas se caracterizan por los colores griseo violáceo a violeta de las láminas juveniles y por sus basidiosporas relativamente grandes en comparación con las de *C. mucosus*. La secuencia ITS es 97% similar a aquellas de las especies más cercanas dentro de la sección *Myxacium* que son *C. collinitus* y *C. mucosus*. El nombre para esta especie nueva es en honor a 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

J. Bot. Res. Inst. Texas 17(1): 259–264. 2023
https://doi.org/10.17348/jbrit.v17.i1.1294

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, Cuphomorphi, 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*.

Table 1. *Cortinarius* sect. *Myxacium*. Sequences included in phylogeny. Newly generated sequenced are in **bold**.

Species	GenBank Accession No.	Collection Number	Location	Vegetation
C. absarokensis	DQ295096	IB19870294	Colorado, USA	*Salix*, alpine
C. brunneoalbus	KR011128	T. Niskanen 09-075	Washington, USA	*Tsuga heterophylla, Abies amabilis"*
C. collinitus (= C. muscigenus)	AY083185	IB19940186	Wyoming, YNP	*Picea engelmannii*
C. glutinosoarmillatus	DQ974720	UC1859790 (src611)	California, USA	*Quercus douglasii*
C. hallingii	KJ421159	JFA11917	Costa Rica	*Quercus*
C. lividoochrascens	DQ295122	IB19960258	Italy	*Quercus cerris*
C. matae	MW508849	JFA 12012	Costa Rica	*Quercus semanii, Q. copayensis*
C. mucosus	AY669591	TUB 011837	Germany	
C. oreomunneae	MW508854	A. Corrales 65	Panama	*Oreomunnea*
C. panamaensis	MW508858	JFA12000	Costa Rica	*Quercus semanii, Q. copayensis*
C. seidliae	KR011125	T. Niskanen 09-132	Washington, USA	*Tsuga heterophylla, Pseudotsuga*
C. trivialis	AJ236066	KH 6	Norway	*Salix nigricans*
C. watsoneae	OP163280	FLAS-F-69145	Florida, USA	*Pinus elliotti*
C. watsoneae	**OQ343666**	K. Liimatainen & T. Niskanen 14-057	Florida, USA	*Quercus virginiana, Pinus* sp.
C. watsoneae	**OQ343667**	DPL-13238	Texas, USA	pine/hardwood upland forest
C. watsoneae	**OQ343668**	DPL-13436	Texas, USA	pine/hardwood upland forest
C. watsoneae	**OQ343665**	DPL-13692 (holotype)	Texas, USA	pine/hardwood upland forest

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), disc sometimes chocolate brown (6F4) or chestnut brown (6F7), 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 fibrillary 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. **Lamella 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.

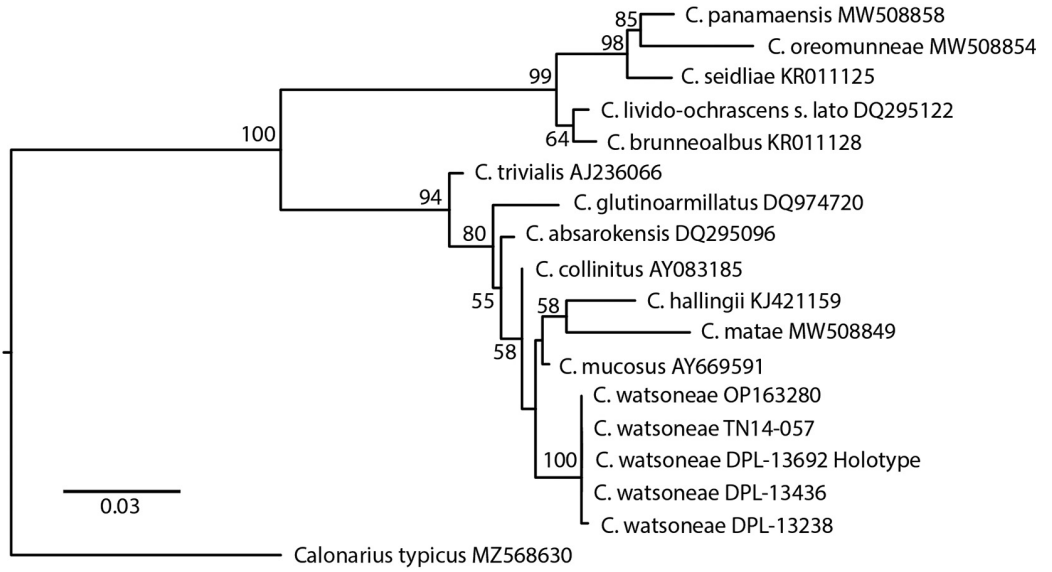

Fig. 1. A phylogram resulting from the RAxML analysis of the dataset. Bootstrap values greater than 50% are indicated above branches.

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.

Additional collections studied: **U.S.A. FLORIDA. Alachua Co.:** Austin Cary Memorial Forest, Slash Pine plantation/in baygall mixed vegetation, with *Pinus elliotti*, and *Serenoa repens* (palmetto), and species of *Vaccinium, Lyonia, Quercus, Persea*, 2 Dec 2021, coll. L. Kaminsky (barcode FLAS-F-69145), GenBank OP163280. **Wakulla Co.:** Crawfordville, 306 Wakulla Beach Road, *Quercus virginiana*, *Magnolia grandiflora*, *Pinus* sp., and *Liquidambar,* sandy soil on calcareous rock, 24 Dec 2014, *K. Liimatainen & T. Niskanen 14-057* (H), GenBank OQ343666. **MISSISSIPPI: Harrison Co.:** Saucier, Harrison Experimental Forest, 30.65, -89.042778, under *Pinus elliottii*, 27 Dec 1986, *W.G. Cibula 1554 with C.L. Ovrebo* (barcode CSU-F-01996, as *C. mucosus*). **TEXAS. Tyler Co.:** Hyatt Lake, Watson Rare Native Plant Preserve, 30.5814. -94.3789, 10 Nov 2018, *DPL-13238* (WTU), GenBank OQ343667; 23 Nov 2019, *DPL-13436* (TAES), GenBank OQ343668; 27 Nov 2021, *DPL*-14164 (TAES).

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. matae* 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.

ACKNOWLEDGMENTS

Thanks to Matt Gordon, Molecular Solutions, for sequencing Texas collections. Appreciation is extended to the Watson Rare Native Plant Preserve for granting permission to collect. Research in Florida was supported in part by a grant from the US National Science Foundation (DEB-1754369, to MES), the NIFA-USDA McIntire-Stennis project 1011527, and UF Plant Pathology Department (FLA-PLP-005289 award). We thank the University of Florida, School of Forest, Fisheries and Geomatics Sciences for providing access to Austin Cary Forest. We thank Dr. Cathy Cripps and an anonymous reviewer for provided helpful comments, and we thank Dr. Juan Luis Mata for writing the Spanish abstract.

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