Cortinarius saniosus Reported from Tropical and Subtropical Florida with Unique Genotype Shared among Midwestern and Southeastern, but not Boreal/Montane, North American Collections

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ABSTRACT

Cortinarius saniosus is for the first time reported from tropical and subtropical Florida, greatly extending the geographic and ecological range of what is otherwise a primarily boreal species. Phylogenetic analysis of these specimens revealed a unique genotype only shared among collections of the species from Florida, Indiana, and Tennessee in eastern North America as well as a more divergent additional subtype restricted to the Florida collections and one of the Indiana collections. Other collections from more northern, boreal North America do not cluster with this genotype which suggests there is some level of divergence between boreal and sub-boreal populations in North America, possibly due to population disjunction caused by glaciation events and switches in ectomycorrhizal hosts. Further investigation into the population structure of this widely distributed species and specifically these tropical and subtropical collections would certainly reveal interesting insights into historical biogeography in this species and serve as a model to compare the evolutionary history of other boreal species with ranges extending south into subtropical and tropical areas. This discovery was a result of the dedicated efforts of non-academic, community mycologists and can hopefully serve as inspiration for others in the community to explore and publish discoveries of their own.

Keywords: *Cortinarius saniosus*, community science, amateur mycology, DNA barcoding, Oxford Nanopore, fungal biogeography

INTRODUCTION

During October 2022, a collection with 5 fruit bodies of an interesting *Cortinarius* species was found growing out of soil under pine (*Pinus* sp.) and southern live oak (*Quercus virginiana*) at Riverbend Park in Jupiter, Florida and was preliminarily identified as *Cortinarius saniosus* based on ITS sequence analysis and morphological examination. Then in January 2023 a second collection was found in Central Florida on the Live Oak Trail (Oak Hammock) at the Lake Woodruff National Wildlife Refuge in De Leon Springs, Florida and later confirmed via Sanger sequencing of the ITS region by J. Ammirati as *Cortinarius saniosus* (Figure 1a,b). This species has not to our knowledge been reported from tropical North America, though it is well documented from boreal and montane North America and Eurasia.

The Florida peninsula is unique in the contiguous United States in containing the only truly tropical ecosystem at the southern end (Udvardy, 1975). As such, Florida is particularly biodiverse and contains unique habitats and endemic biodiversity, though the mycological natural history remains somewhat poorly documented. Historically, Florida has been hypothesized to play an important role as a glacial refugium during past glaciation events and is currently considered a biodiversity hotspot (Soltis et al., 2006; Lyman & Edwards, 2022).

Cortinarius saniosus is a species in section *Saniosi* of subgenus *Telamonia* considered relatively common and widely distributed in Europe where it often is reported associating with *Salix* but sometimes with other deciduous trees (see Lindström et al., 2008; Kokkonen 2020). This species appears to be highly variable in pileus coloration ranging from blackish-brown, dark yellow brown, reddish brown, to orange brown, likely leading to the species having been described many times (Liimatainen et al., 2020). It has previously been reported in North America (again, often, but not always, associated with *Salix*) from the Pacific Northwest (Harrower et al., 2011), the Rocky Mountains (Moser & McKnight, 1987; Moser, 1993), California (Moser, 1993), Eastern Canada (Landry et al., 2021), and was recently reported from temperate South America (Chile) growing with introduced *Salix babylonica* (Palfner et al., 2023). See Lindström et al., 2008 for a thorough description of the history of the species and its name.

While the morphology generally matched descriptions of *C. saniosus* and the ITS region demonstrated a high level of similarity, multiple name and type species showed homology with

the sequences generated here. In order to determine if these collections are indeed this species, the ITS region of two of the specimens were sequenced and compared with all other available specimen-based and environmental sequences. Molecular methods such as DNA sequencing have become increasingly popular for fungal identification in recent years, offering a rapid and accurate method to confirm identifications based on traditional methods. Identification of species based on DNA similarity can also be performed, but should be performed carefully in the absence of additional context (Hofstetter et al., 2019). The definitive work to date on subgenus *Telamonia* (Liimatainen et al., 2020) has shown the ITS region to be highly effective at species determination though differences between species can be as few as 1 bp while other species can exhibit >1 % variation.

MATERIALS AND METHODS

Collection and Morphological Analysis of Samples. The fruiting bodies were photographed in habitat using a Nikon d70 and focus stacked using the program ZereneStacker as well as an iPhone 12 to grab the geolocation. The fruiting bodies were then dried partially in a tackle box on the dashboard of a car and finished in a dehydrator at approximately 130 °F. For microscopic study, a single lamella was removed from each collection and mounted in 5% KOH. Spores were then selected based on maturity (more coarsely ornamented, darker basidiospores) and 30 measurements were taken for each collection indicating length and width (profile view). The collection from Central Florida (MO 514955) has been deposited at the University of Washington, Burke Museum Herbarium (WTU) (accession number pending) and the original collection from South Florida (MO 507581) will be deposited at University of Florida, Gainesville (FLAS, accession number pending).

DNA Extraction, PCR amplification, and Sequencing. The PCR amplification was performed by one of the authors (KC) of Ohio Mushroom DNA Lab (OMDL) and sequenced by MCLab, South San Francisco, California. The DNA of the *Cortinarius saniosus* specimen (MO507581) was extracted with NaOH and a standard Tris TAE buffer and PCR was performed using ITS1f and ITS4 primers. The DNA was amplified using the 8 well MiniPCR thermal cycler following the methods of Jakob (2023). A second observation (MO514955) was discovered three months later and was sequenced both by Sanger (by Joe Ammirati, University of Washington) and

Nanopore technologies (see below).

For the Nanopore sequence the extraction, PCR amplification, and sequencing steps for specimen MO514955 were performed by the same author (KC) at the OMDL following the protocol of Russell (2023a). In brief, X-AMP DNA reagent was used to extract DNA and the ITS was amplified using a GeneAmp PCR System 9700 using ITS1f and ITS4 primers. Afterwards a PCR cleanup was conducted using magnetic beads. Lastly sequencing was done using a System 76 Laptop and the Oxford Nanopore MinION device with the Flongle attachment.

Phylogenetic Reconstruction from Specimen-Based ITS Sequences. All ITS sequences from specimens identified as belonging to *Cortinarius* subgenus *Telamonia* sect. *Saniosi* (based on Liimatainen et al., 2020; Kokkonen, 2020) were downloaded from GenBank (Sayers et al., 2023). Additional sequences from unidentified or ambiguously identified specimens that had BLAST (Altschul et al., 1990) results at >95% similarity and >80% coverage were included in this first data set. One specimen each of *C. mallaensis* and *C. fusisporus* var. *olivaceodepressus* were included as outgroups (Supplementary file 1).

The sequences were aligned using Muscle (Edgar 2004) in MEGA 11 (Tamura et al., 2021), searched for aberrant sequences to remove, and trimmed to remove the flanking small subunit and large subunit regions (Supplementary file 2). No additional regions were removed from the ITS region. A Maximum Likelihood phylogenetic reconstruction was performed using GTR+gamma+i parameters and subjected to 500 bootstrap replicates.

After initial analysis and reclassification of misidentified or ambiguously identified sequences, a second refined alignment and phylogenetic analysis was performed as above except the dataset was limited to sequences from specimens of *C. saniosus* with one sequence each of *C. subsaniosus* and *C. gentillissimus* as outgroups (Supplementary file 3).

Analysis of Environmental Sequences. All sequences within a 1.5% similarity threshold of *Cortinarius saniosus* were filtered in UNITE (SH Code: SH1283128.09FU; Kõljalg et al 2020) and downloaded through the PlutoF portal (Abarenkov 2010) to search for additional specimens matching this temperate and tropical North America genotypes. This dataset consisted of all specimen-based sequences as well as ITS sequences generated from soil samples world-wide. No

sequences from soil samples in temperate North America were present in this dataset. In total, 1436 sequences were downloaded, aligned with the target specimen-based sequences as above, and manually compared for matching genotypes (Supplementary file 4).

RESULTS

Morphological Examination. The Florida collections' macromorphology matches descriptions of the *C. saniosus* from recent literature (Lindström et al., 2008; Kokkonen, 2020; Landry et al., 2021) in the following characteristics: *Cap*: conical to campanulate that depresses around a distinct umbo with age, hygrophanous, light to dark brown or a light orange-brown in color. *Stipe*: somewhat clavate, orange-brown to light brown and becoming dark when handled. *Gills*: close to subdistant, broadly attached. Some notable differences are in the Lindström et al., (2008) collections: it is noted to be "Seldom with striae from translucent gills (but can be visible up to half the distance to centre" while the Florida collections seem to be consistent with translucent striations that run about 3⁄4 of the way to the umbo. The spectrum of variation in color doesn't seem to be represented by the Indiana, Tennessee, and Florida collections reported in the other sources but appear rather uniformly light orange-brown in color when moist.

Spores of the two collections from Florida had spore dimensions that were markedly longer compared to Kokkonen (2020) whereas the widths matched closely with both descriptions though there was variance within the two Florida collections and the two published descriptions (Figure 1c, Table 1).

Specimen-Based ITS Dataset and Phylogenetic Reconstruction. The initial tree recovered from the alignment of the entire section *Saniosi* of subgenus *Telamonia* showed that many sequences were not identified based on the latest taxonomic species concepts in this group (Figure 2). Some species reported as close to *C. saniosus* did not cluster in the core section *Saniosi* but rather with the outgroups and may not belong to the section as currently circumscribed (*C. vienoi* and *C. perzonatus* and one of the ex-type sequences of *C. rufoanuliferus*).

Of particular interest are two ex-holotype ITS sequences of *Cortinarius rufoanuliferus.* One (MN841170 generated by Kokkonen, 2020), is a 100% match to *C. parvanulatus* under which it is listed as a synonym while the other, (MT935287 generated by Liimatainen et al.; 2020) is

clearly in the cluster of *C. saniosus* sequences. This raises the distinct possibility that the type collection is a mixed specimen and highlights that care should be taken even when comparing results to sequences based on type specimens.

Table 1. Comparison of spore dimensions between the Florida collections and those in recent literature on this section.

Source	Length Min	Length Mean	Length Max	Width Min	Width Mean	Width Max	Q-Value Min	Q-Value Mean	Q-Value Max
MO 507581	8.2	9.21	10.34	5.06	5.6	6.05	1.45	1.64	1.96
MO 514955	8.45	9.4	10.63	4.88	5.75	6.81	1.42	1.63	1.78
Lindström et al., 2008	8.1	-	10.1	5.0	-	6.6	1.5	-	1.7
Kokkonen, 2020	7.5	-	8.5	5	-	6	-	-	-

In the *Cortinarius saniosus* phylogeny, a unique lineage of temperate to tropical eastern North American specimens is recovered containing the two Florida collections along with one Tennessee and three Indiana collections (Figure 3). These collections all shared one unique single nucleotide polymorphism and a subset of collections (the two Florida collections and one of the Indiana collections) shared multiple additional unique polymorphisms. The shared genotype of the Indiana collections with the Florida collections compared with more northern boreal and montane collections of *C. saniosus* from North America (Pacific Northwestern, Rocky Mountains, and Eastern Canada) and supports the hypothesis that Indiana and Florida fungal species assemblages are more similar than Indiana and boreal Canadian communities (Russell, 2023b).

When comparing the ITS sequences of specimen MO 514955 generated using Sanger (OR351068) and Oxford Nanopore (OR540735) only one difference was found in position 59 (Supplementary file 4) where the Sanger sequence recovered one T residue while the Oxford

Nanopore consensus sequence reported two T nucleotides, a feature unique to this sequence among all the sequences. Further analysis revealed that the reads in consensus contained either one (33 reads) or three (18 reads) T nucleotides in the majority of the reads (three had other numbers of T nucleotides), potentially representing two different haplotypes (see Supplementary file 5). The current Nanopore processing pipeline built a consensus sequence with two T nucleotides, a motif not present in any of the raw reads in consensus. This is a known issue of the Nanopore consensus software and has been referred to as "haplotype conflation" (S. Russell, personal communication).

Analysis of Environmental Samples. The non-boreal North American genotype, represented by the collections from Florida, Tennessee, and Indiana, is defined by a single nucleotide polymorphism C to T mutation at position 147 that was not found in any of the 1436 sequences in the UNITE dataset composed primarily of environmental samples across the boreal zone (though also extending into temperate Asia) (Supplementary file 4). An additional unique subtype found in the two Florida collections and one of the Indiana collections (Plischke) was defined by a 5-T repeat at positions 506-510 in ITS2 and a (though a few had one additional T nucleotide) T to C mutation at position 44 (Supplementary file 4). While poly-T repeat length variation is typically not considered to be particularly phylogenetically informative as it commonly occurs due to polymerase slippage *in vivo* during DNA replication or *in vitro* during PCR amplfication, the fact that this repeat length was detected across multiple specimens and sequencing technologies demonstrates that this is a biologically real and useful difference. These polymorphisms present in the ITS sequences of temperate eastern North American specimens were not found in any sequences in the large environmental dataset with representation across a large geographic range, supporting that these are consistent autapomorphic characters of a divergent population.

DISCUSSION

The range extension of *Cortinarius saniosus* into tropical Florida is surprising given the otherwise boreal to montane distribution of the species in North America, Europe, and Asia and has not been previously reported to our knowledge. Sequence data is available for just a few scattered North American collections in the eastern United States (i.e., Indiana, Tennessee) and

is to be expected in other states in this range. There are only 15 specimens on iNaturalist (on 9/12/2023) with observations from the west coast of North American and Arizona in addition to the previously mentioned eastern collections. It is likely that the species is more common but that collections of *Cortinarius* subgenus *Telamonia* are not commonly identified to species given the difficulty in identifying species in this group without molecular data. Further sampling will be necessary to confirm the distribution of the genotype reported for these collections. Additional population genetic research as well as sequencing of other regions with high rates of nucleotide substitution would help elucidate the evolutionary biogeography of the species in this region.

This expansion of distribution was uncovered as a direct result of efforts made by non-academic (amateur) mycologists by combining molecular, morphological, and ecological data as well as utilizing modern online resources such as iNaturalist and Mushroom Observer. This uptick in community/amateur science is only increasing in popularity and with more avenues for DNA barcoding emerging such as Stephen Russell's Continental MycoBlitzes, FunDiS supported projects, and the Ohio Mushroom DNA Lab free DNA sequencing efforts, it is likely our understanding of the distribution of many more taxa will expand (Cantonwine et al., 2022).

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SUPPLEMENTARY FILES

Supplementary File 1.
https://drive.google.com/file/d/1oZpJa5QZJUEO3WAlu6ezWcPr1VpR6dqO/view?usp=sharing

Supplementary File 2.

https://drive.google.com/file/d/1WCkXKT8Zhxg6iKGB13Df_y38j9rvFsNG/view?usp=sharing

Supplementary File 3. https://drive.google.com/file/d/1QsaZzjx4ltoa2oMGQTocX-0aF4aIHS_r/view?usp=sharing

Supplementary File 4. https://drive.google.com/file/d/19XZj8A1Ks9nmeyLjY4zWvFn__Ppt3XV8/view?usp=sharing

Supplementary File 5. https://drive.google.com/file/d/1HaQcbm4EbeFssiEWev0qR2dXV-ZirbCj/view?usp=sharing

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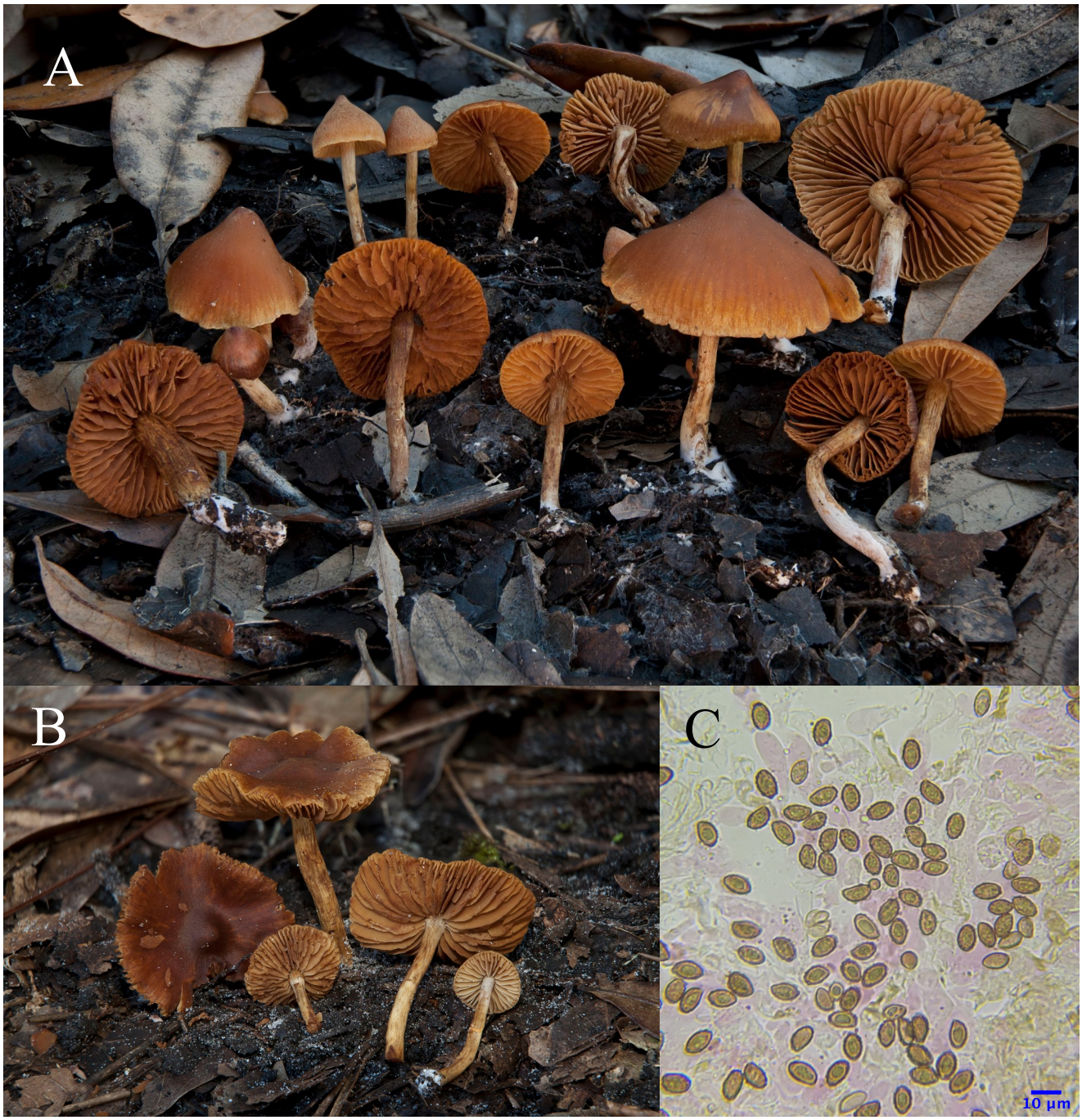

Figure 1. Photos of the fruiting bodies and basidiospores of *Cortinarius saniosus* from Florida A. De Leon Springs, Florida (MO 514955) B. Jupiter, Florida (MO 507581) C. Spores from collection MO 507581 at 1000× magnification. (Photos by S. Ostuni)

Figure 2. Phylogenetic reconstruction of *Cortinarius* subgenus *Telamonia* section *Sansiosi* based on ITS sequences on species identified as belonging to the section or otherwise showing high sequence similarity. Collections with the North American non-boreal genotype are highlighted.

Figure 3. Phylogenetic reconstruction of *Cortinarius sansiosus* based on ITS sequences. Collections with the non-boreal/montane genotype are highlighted.