Mycena jingyinga: a recently described species from Taiwan is reported in North America

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ABSTRACT

Mycena jingyinga, reported here for the first time in North America, is otherwise only known from eastern and southeastern Asia. The North American distribution of *Mycena jingyinga* is unclear; it may be this species was recently introduced, or it may have a broad distribution but has gone unreported. Several closely related species have a wide geographic distribution spanning continents.

Keywords: *Mycena jingyinga*, fungal biogeography, community science, Oxford Nanopore.

INTRODUCTION

An unrecognized *Mycena* species with a coarsely fibrillose tufted stipe, viscid, pale gray pileus, and clustered growth habit was found on herbaceous debris on May 20, 2023 by Crystal Davidson in Clermont County, Ohio. The distinctive appearance does not match species of *Mycena* section *Fragilipedes* reported from temperate North America or Europe (Smith 1947, Giovanni 2006). *Mycena*, a genus of saprophytic fungi, contains about 500 currently accepted species as of the last estimate (Kirk et al., 2008).

MATERIALS AND METHODS

Molecular Analysis. DNA extraction, PCR, and Oxford Nanopore sequencing was performed by one of the authors of the Ohio Mushroom DNA Lab (OMDL) following the protocol of Russell (2023a). The DNA was extracted from a dried sample of *Mycena jingyinga* (Genbank OR572512) with X-AMP DNA reagent (IBI Scientific). Targeting the internal transcribed spacer (ITS) region using ITS1F and ITS4, the PCR was done using a GeneAmp 9700 thermal cycler. The final sequencing step was done with the ONT MinION following the protocol of Russell 2023. In order to determine the species identity, molecular and morphological characteristics were used, and the DNA sequence was compared against similar species using NCBI BLAST as well as a local BLAST search using Stephen Russell's MycoMap database.

Phylogenetic Reconstruction. All ITS sequences from species with BLAST (Altschul et al., 1990) results >96% similarity and >70% coverage were included in the alignment (Supplementary File 1). Additional sequences from representative species matching the criteria above are included. The sequences were aligned using Muscle (Edgar 2004) in MEGA 11 (Tamura et al., 2021), visually inspected to look for aberrant sequences to remove, and trimmed to remove the flanking small subunit and large subunit regions (Supplementary File 2). No other regions were excluded from the analysis. A maximum likelihood phylogenetic reconstruction was performed using GTR+gamma+i parameters and subjected to 500 bootstrap replicates.

Micromorphological examination. Unfortunately, the specimen was rather damaged during transport but sufficient material for microscopic examination was present. Sections of the pileus, lamellae, and stipe were rehydrated in 2% KOH or 2% KOH with Congo Red and examined at 400× and 1,000× magnification. Thirty spore measurements were made using ImageJ (Schneider et al., 2012), the Q-values (length/width) were calculated, and the Min-Mean-Max (excluding the most extreme values) are reported.

RESULTS

Molecular analysis. The ITS region was found to be identical to the sequence from the Holotype of *Mycena jingyinga*, (Genbank MG324365.1) a species with bioluminescent mycelium recently described from Taiwan (Chang et al., 2020). A strongly supported unique clade was recovered

that included the Ohio specimen, the ex-holotype sequence of *M. jingyinga* along with its paratypes, the ex-holotype sequence of *M. adnexa* (a later synonym of *M. jingyinga* published one year later; Tolgor et al., 2021), and several sequences identified as *M. abramsii* (Figure 2). In our dataset, one misidentified sequence, labeled as *Mycena abramsii* (MN294837.1), was excluded as it represents a species of *Coprinellus* section *Micacei*. Our sequence is the only vouchered specimen of the species from outside of eastern and southeast Asia. Interestingly, a 359 base pair Illumina sequence from a stool sample in Texas (KY935124) matched the Ohio collection as well as the type sequence. Given that *Mycena jingyinga* is not expected to be a part of the human gut-flora, it's likely to be a contaminant from the ambient environment. The species appears phylogenetically close to numerous likely species level clades containing sequences identified as *M. abramsii* across broad geographic distributions. Several other clades tentatively identified with *M. fragillima/murina/*sp. PNW10 (western North America, possibly also Europe and East Asia) *Mycena* sp. "IN26" (western and eastern North American and Europe), and *Mycena* sp. "IN21" as well as *M. venus*, another bioluminescent species described alongside *M. jingyinga* (Chang et al., 2020).

Macromorphological analysis. Pileus up to 5 mm broad, narrowly convex becoming broadly convex with age, with a slight umbo, margin decurved to straight, crenulate, abruptly pale neutral gray at disc, nearly white outward, translucent striate when moist to disc, context very thin. Lamellae: adnate, relatively narrow, close with (0-) 1 tier of lamellulae, white. Stipe up to about 10 mm long and 1.5 mm broad, central, curving from the base in a wide arch, terete, base slightly clavate and gradually tapering upward, very pale gray near base, white by about one quarter to one third of the way, densely but minutely floccose, ornamentation more concentrated toward base. Odor like bleach. Cespitose-imbricate to subconnate in pairs on a small herbaceous stem.

Micromorphological analysis. Spores ellipsoid to slightly narrowed toward apiculus, thin-walled, smooth, heterogenous or with several small guttulae, rarely uniguttulate, 7.6–8.8–10.2 × 4.2–4.9–5.5 µm, Q-Value 1.50–1.81–2.04. Cheilocystidia: fusiform, lageniform, to sublanceolate, sometimes irregularly inequilateral, apices subacute to subobtuse, rarely diverticulate, sometimes strangulate when particularly attenuated, some short, narrowed base while some others have a long, attenuated base, 28–49.5 × 5.5–10 µm. Pleurocystidia: absent. Pileipellis: a cutis of repent hyphae 3–4.5 µm broad, with short thick (up to 4.5 µm broad) and knobby to sometimes

ascending and multiple branched excrescences. Stipitpellis: 3–4.5 µm broad, with scattered, short, broad simple excrescences, 3.5–4 × 2.5–3 µm. Caulocystidia: much like the cheilocystidia, few observed. Clamp connections: present.

DISCUSSION

The macromorphology closely matches that of the original description (Chang et al., 2020) with two notable discrepancies. The stipe in the original is described as pubescent while this specimen exhibits a stipe with a denser, nearly floccose surface. Additionally, this specimen had a bleach-like odor while the odor in the original description was listed as absent. Odor can be influenced by environmental conditions as well as the subjective experience of the documenter so we do not give much weight to this difference but further study may shed light on odor variability within species of *Mycena*. The micromorphology is quite congruous with the following notes: the Ohio collection had spores on the larger end of the reported range and the cheilocystidia were shorter but significant variation and the length of the attenuated base extending past the lamellar edge was noted. The mycelium of *M. jingyinga* is shown to be bioluminescent in the original description but this was not looked for in the Ohio collection.

We propose for consideration the common name “Crystalline Mycena” for this mushroom which is a rough translation of the Chinese 晶莹小菇 (“Jīngyíng xiǎo gū”) which refers to crystal clear quality and small size of the fruiting body. The common name is proposed according to the form, guidelines, and philosophy of Kalichman 2023.

While such a disjunct distribution is unexpected (otherwise known from Japan, South Korea, China, and Vietnam based on ITS sequences), multiple clades contain sequences from specimens collected in multiple continents implying that intercontinental distributions may be common in this group if the ITS phylogeny recapitulates species-level relationships. There are two explanations for this surprising find: *M. jingyinga* has been recently introduced, likely along with ornamental plants, from Asia or it has a natural distribution in North America but has not been found or identified until now, possibly due to the difficulty of identifying *Mycena* species.

Several closely related species-level clades are primarily composed of specimens labeled *M. abramsii*, a name originally published from California but used widely in Europe and Asia but

without a certain identity based on this analysis. Further investigation, including obtaining a DNA sequence from Murrill's type, would help clarify the taxonomy and nomenclature of this group. The dataset and alignment used in this paper is provided for future analysis (Supplementary files 1 and 2).

Emerging community mycology DNA barcoding efforts have recently been highly successful at uncovering interesting and unknown macrofungal biodiversity (Cantonwine et al., 2022) and we anticipate many more as ongoing projects continue.

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SUPPLEMENTARY FILES

Supplementary File 1

https://drive.google.com/file/d/14GWHA0hgPdGXz7EiAimSoZoarTQcECCR/view?usp=drive_link

Supplementary File 2

https://drive.google.com/file/d/1xw7jE8W0ir0y4T34WI2cpkE7khVt-lDs/view?usp=drive_link

https://drive.google.com/file/d/1xw7jE8W0ir0y4T34WI2cpkE7khVt-lDs/view?usp=drive_link

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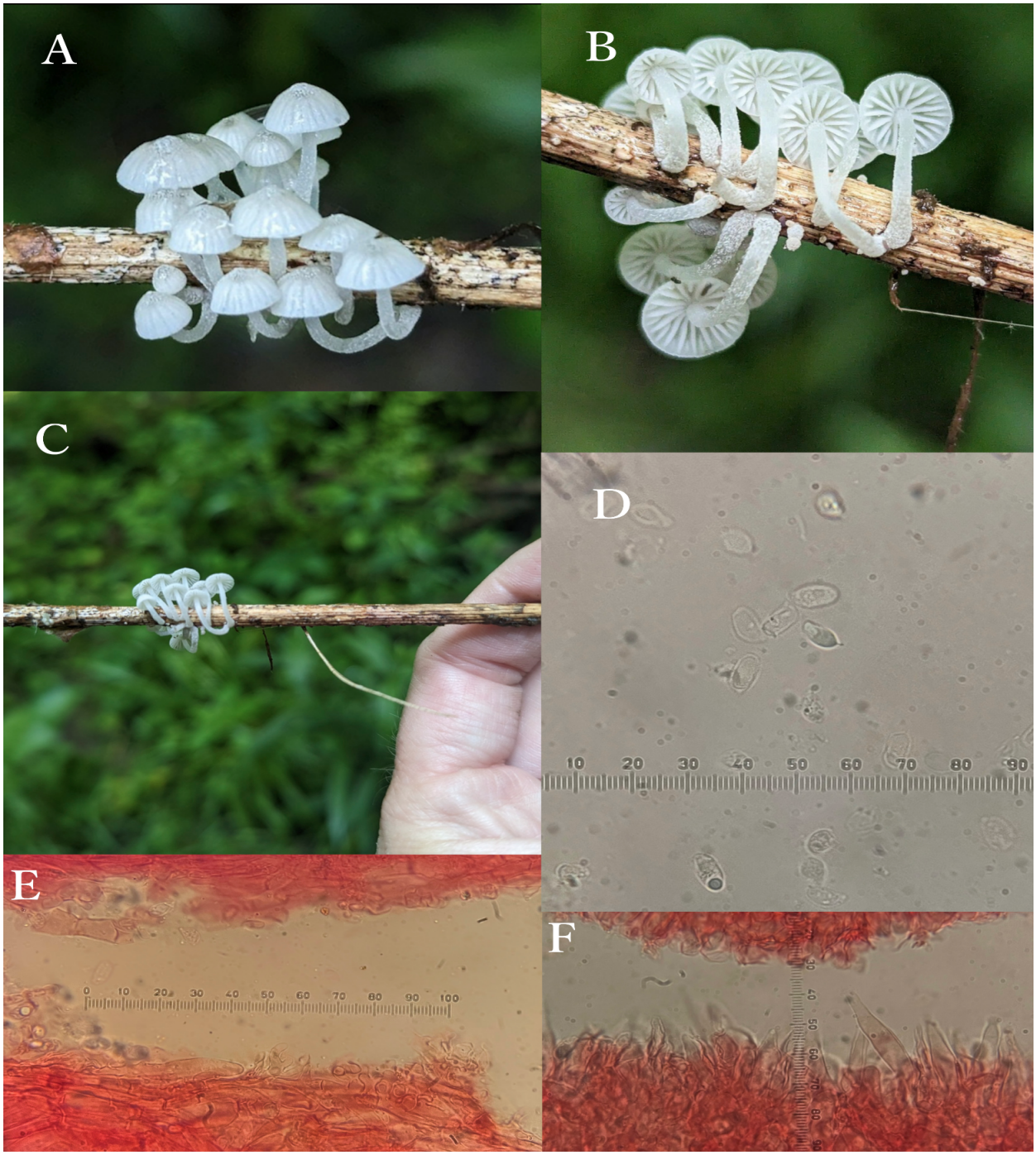

Figure 1. Fruiting Body and Micromorphology of the Ohio collection of *M. jingyinga*. Micrographs at 1000 × magnification and 1.1 µm per division. A,B,C. Fruiting bodies in situ. D. Spores in 2% KOH. E. Pileipellis in 2% KOH with Congo Red (top and bottom). F. Cheilocystidia in 2% KOH with Congo Red.

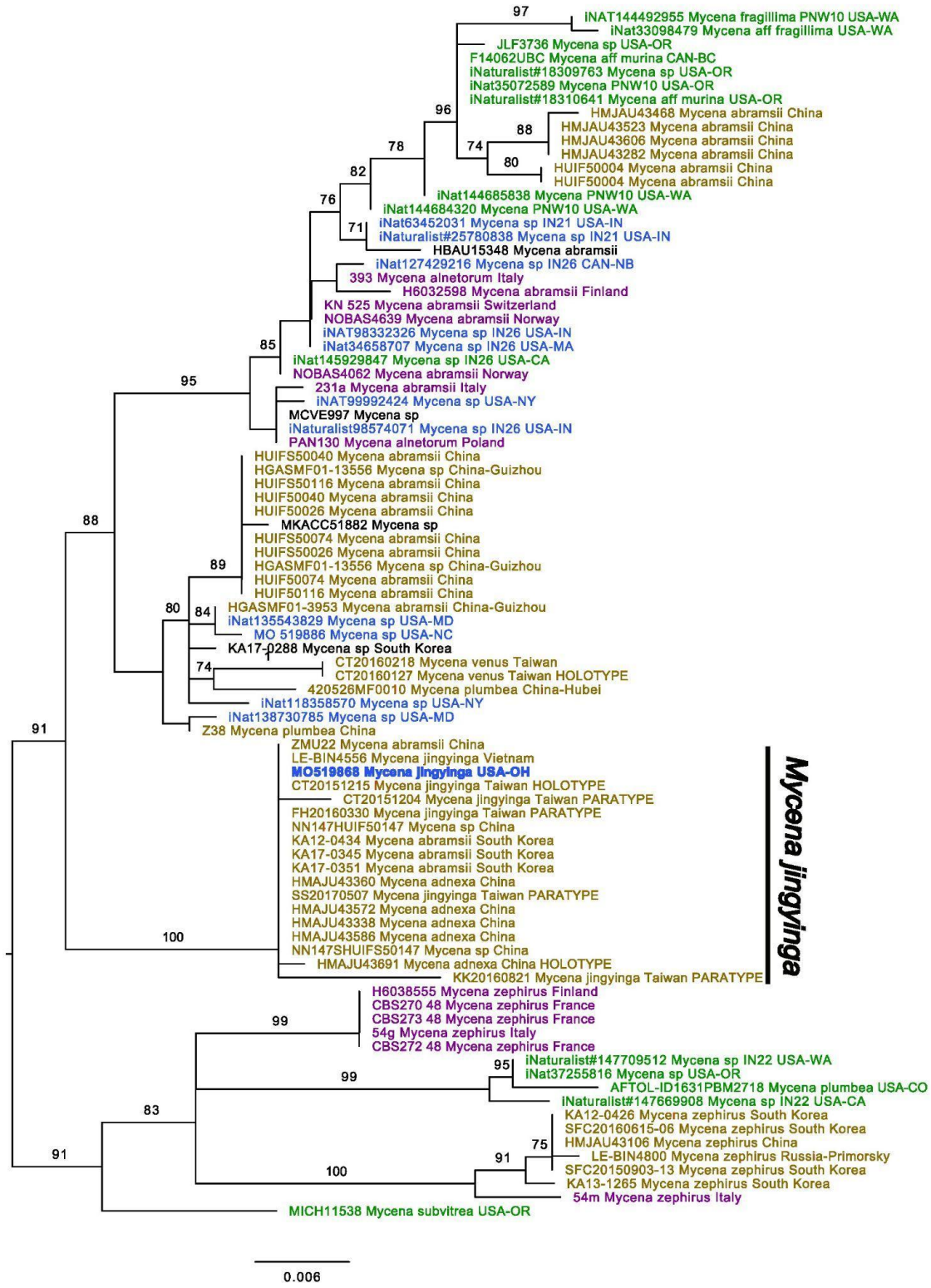

Figure 2. Phylogeny of *Mycena jingyinga, M. abramsii,* and other related species. The collection described in this paper is in bold. Taxa are color-coded by geographic region as follows: Blue - Eastern North America, Green - Western North America, Orange - East Asia, Purple - Europe