Psilocybe caeruleorhiza: a new, cold weather fruiting species of psilocybin containing mushroom from the midwest in section *Aztecorum*

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ABSTRACT

Psilocybe caeruleorhiza is described as a new, bluing, wood-rotting species from the midwestern United States fruiting in the late fall and winter. Morphological features place it in *Psilocybe* section *Cyanescens* or *Semilanceata* but near section *Aztecorum* based on micromorphological characters (i.e., ellipsoid to subellipsoid spores and ventricose-rostrate to langeniform cheilocystidia) while it most closely resembles *P. ovoideocystidiata* macroscopically. This new species can be distinguished from similar species by its stature and thin, subcortinate partial veil which leaves no annulus. Multigene phylogenetic analysis (nrITS, nrLSU, rpb1, rpb2, tef1) supports the novelty of the species and placement in section *Aztecorum*. A full description is provided along with photographs of fresh specimens and phylogenetic analysis.

Keywords — Basidiomycota, Agaricales, Hymenogastraceae, 1 new taxon, Community Science

INTRODUCTION

In November, 2021 a blueing mushroom growing in wood chips was discovered in Iowa City, Iowa and posted on iNaturalist (iNat); a state where historically this genus was not known to occur. Simultaneously it was also found in the state of Pennsylvania. This was then followed by observations in Ohio and Indiana in December of 2021. In the subsequent years this species was found multiple times in different locations throughout Indiana and Ohio; all growing on wood chips in disturbed areas (Figure 1). Initial ITS barcoding similarity suggested this species shared affinity with *Psilocybe* sect. *Aztecorum* Guzmán (*P. aztecorum* and *P. serbica*).

Figure 1. Fruiting bodies of the holotype collection (iNat 194406876) of *Psilocybe caeruleorhiza* in situ.

These collections did not adequately match species of *Psilocybe* previously recorded in the midwestern United States; *P. ovoideocystidiata* Guzmán & Gaines, *P. caerulipes* Peck, and *P. cyanescens* Wakefield. This species has been provisionally referred to as *"Psilocybe caeruleorhiza* nom. prov." (Rockefeller, 2022) as well as "*Psilocybe* sp-PA01" (Russell, 2022). In order to resolve the identity and taxonomic placement of this presumptive new species, molecular and microscopic investigation was performed and is presented here.

MATERIALS & METHODS

Collection and macromorphological examination. Macromorphology was described from field-fresh collections using Mushroom Observer Glossary of Mycology Terms (https://mushroomobserver.org/glossary_terms) and Easy Guide to Mushroom Descriptions (Scates 1982). Colors are reported based on general common usage and not based on a specific color nomenclatural reference. Minimum and maximum measurement outliers are given before (minimum) or after (maximum) the reported range. Specimens were dried at 140 degrees F using a food dehydrator. Herbarium abbreviations follow Thiers (continuously updated).

Micromorphological examination. Thin hand sections of the holotype were rehydrated and mounted in 5% KOH or glycerol. Characters are described based on the vocabulary of Largent (1977). Measurements were made using the free micrograph processing software ImageJ (Schneider et al., 2012). Spore measurements were made in profile view. Dimensions are given in the following format: (min outlier) 10 percentile–mean–90 percentile (max outlier), N=sample size. Q (quotient)-value is the length divided by the width.

PCR and DNA Sequencing. Dried material of the paratype was sent to ALVALab (Oviedo, Spain) for DNA extraction, PCR, and sequencing. Sequences of the nrITS (nuclear ribosomal Internal Transcribed Spacer) region were generated using ITS1F and ITS4 primers (Gardes & Bruns, 1993; White et al., 1990). For the nrLSU (nuclear ribosomal Large Subunit), LR0R and LR5 primers (Vilgalys & Hester, 1990) were used. For rpb2 (RNA polymerase II gene, second largest subunit), the 7R2 primer was used (Liu et al., 1999). For rpb1 (RNA polymerase II gene, largest subunit), RPB1AF (Stiller & Hall, 1997) was used. For tef1 (Translation Elongation Factor 1), the 1567R primer (Rehner & Buckley, 2005) was used. Additional paratype ITS sequences were generated by Mycota Labs, The Ohio Mushroom DNA Lab, and Counter Culture Labs following the methods outlined in (Russell, 2023; Canan et al., 2024).

Phylogenetic Analysis. All sequences (excluding environmental sources and genomes) belonging to the genus *Psilocybe* were downloaded from GenBank (Sayers et al., 2023) on 12/1/2023 and organized by taxon and specimen voucher. Sequences generated in the study were included to populate the dataset. Each region was aligned using MAFFT v. 7 (Katoh & Standley, 2013) in UGENE (Okonechnikov et al., 2012) and preliminarily analyzed using a maximum likelihood phylogenetic analysis in raxML GUI 2.0 (Edler et al., 2021) using RAxML-NG (Kozlov et al., 2019) . Based on these preliminary results, misidentified, misclassified, low quality (high proportion of polymorphic sites or highly gappy), and specimen-duplicate sequences were excluded from the dataset. For the final analysis, a curated dataset of only sequences that clustered most closely with *Psilocybe* section *Aztecorum* and select representatives from other clades with multilocus coverage were included in the final dataset (Supplementary File 1). Each region was aligned separately as above, and then concatenated (Supplementary File 2). The best-fit model was selected and assigned to each partition (codon positions and introns analyzed separately) using ModelTest-ng v0.1.7 (Darriba et al., 2020) based on AICc (Supplementary File 3). The concatenated, partitioned dataset was analyzed on 20 random starting trees with transfer bootstrap estimation using the autoMRE option to determine the number of bootstrap samples required. Phylogenies were visualized, annotated, and edited in FigTree (Rambaut 2023).

TAXONOMY

***Psilocybe caeruleorhiza* K. Canan, Ostuni, Rockefeller, and Birkebak, sp. nov. MB# 853101, Figures 1-4**

Etymology: From the latin *caeruleo* (adjective) meaning blue and greek *rhiza* (noun) in reference to the strongly blue mycelium that can be seen during growth on solid agar media. Note: while ICN article 23A.3(c) (Turland et al., 2018) recommends against proposing epithets combining two languages, the common usage of this name as a nom. prov. justifies a departure from said recommendation.

Common Name: We would like to propose the common name “Winter Teachers” for this species (referring to the time of year and region that it fruits)

Holotype: USA: Ohio, Hamilton County, Montgomery, Johnson Nature Preserve, 39°15'36.3"N 84°21'22.6"W, elevation 253 m, December 16 2023, Leg. K. Canan, Ohio State University Herbarium (OS - accession number pending) / iNat 194406876. Isotype: University of Florida Hwerbarium (FLAS-F-72663).

Paratypes: USA: Ohio, Hamilton County, Montgomery, Johnson Nature Preserve, 39°15'36.3"N 84°21'22.6"W, elevation 253 m, December 2 2021 (iNat 102406598 / FLAS-F-72649), USA: Indiana, Marion County, Indianapolis, 1200 Madison Avenue, 39°45'05.2"N 86°09'17.4"W, elevation 219 m, December 16, 2021 (iNat 103203179 / MYCO-AU111), USA: Ohio, Scioto County, Valley Township, Rest Area, 38°56'60.0"N 83°01'29.9"W, elevation 170 m, December 11, 2022 (iNat 144201103), USA: Pennsylvania, Allegheny County, Pittsburgh, November 17, 2021 (iNat 160500191 / MO 488261), USA: Kentucky, Jefferson County, Louisville, December 22, 2022 (iNat 144891121), USA: Iowa, November, 2021 (iNat 106753938), USA: Indiana, Marion County, Lawrence, elevation 254 m, January 1, 2024 (iNat 195504414 / PULF29800).

Phenology: So far observed fruiting between October and January. Most commonly encountered in December.

Habit, Habitat, & Distribution: gregarious to cespitose-imbricate on dyed mulch, has been found in Ohio, Indiana, Pennsylvania, Kentucky, and Iowa.

Description

Pileus: 15-80 mm broad, convex to more-or-less hemispherical when young, typically with a slight to moderate obtuse umbo and an incurved to almost inrolled, becoming convex to pulvinate with a slight umbo or plane to even slightly depressed at center when mature, slightly to distinctly uneven and lobed at all ages, margin variable at maturity from incurved to upturned, often lobed and split, rusty orange to copper to orange brown when moist, hygrophanous, fading to yellowish brown to olivaceous buff, sometimes mottled with innate, darker streaks, bruising bluish-green with handling or in old age, smooth, lubricous when moist, often wrinkling with age, margin sometimes with a thin band of whitish veil remnants when young, often disappearing in age. Context off-white, bruising bluish. ***Lamellae:*** adnate to sinuate, sometimes with a decurrent tooth, relatively broad, close, typically with 3 lamellulae between lamellae, buff to drab when young, become lilac tinged medium brown to purplish brown at maturity. ***Stipe:*** up to

60 mm in length, central to slightly eccentric, equal to slightly tapered toward middle, off-white to dull straw-colored to tan, bruising blue upon handling, with white pruinose bands or fibrillose patches upward, appressed fibrillose to silky below, sometimes splitting with old age when dry, base with copious rhizomorphs. Context white to pale ochraceous, fibrous-pithy. ***Partial Veil:*** thinly membranous, splitting to appear nearly cortinate, white to pallid, evanescent or leaving white, fibrillose-oppressed remnants on or near the pileal margin. ***Odor:*** farinaceous. ***Spore Print:*** purple-brown.

Figure 2. Fruiting bodies of the holotype collection (iNat 194406876) of *Psilocybe caeruleorhiza.* In situ B, D or on black velvet (A, C). (Photography: Kyle Canan; Photo processing: Scott Ostuni).

Figure 3. Fruiting bodies and culture of *Psilocybe caeruleorhiza.* A) iNat 160500191 (Paratype); mycelium on agar media displaying blue rhizomorphs B) iNat 102406598 (Paratype); in situ. C) iNat 102406598; photographed in hand D) iNat 160500191 (Paratype); in situ E) fruit bodies at holotype location 2-3 days prior to collection of specimen iNat 194406876 with ruler for size reference. (Photography: A: Alan Rockefeller; B, C: Kyle Canan; D: James R; E: iNat User: squirrely)

Basidiospores (10.2) 10.9–12.1–13.2 (15.1) × (5.5) 5.9–6.4–7.1 (7.9) µm, Q-value (1.67) 1.73–1.88–2.04 (2.18), N = 40 (measurements from deposit on pileus surface), ellipsoid to subellipsoid to slightly asymmetrical ("mango-shaped" per Guzmán), thick-walled, walls ≥0.5–<1.0 µm with an apical germ pore, dark to medium brown. ***Basidia*** 30.6–48.9 × 7.4–9.5 µm, subcylindrical to slightly clavate, typically with a broad base, sometimes slightly medially constricted, hyaline, sometimes malformed with brown encrustations, thin-walled, typically 4-spored, rarely 2-spored sterigmata straight to slightly cornute, narrowly conical, up to 4.5 µm long. ***Basidioles:*** 21.5–32.4 × 7.1–8.6 µm, subcylindrical to subclavate, hyaline, thin walled. ***Hymenium:*** Individual elements appearing more-or-less hyaline though Faintly pigmented in masse. ***Pleurocystidia:*** Very rare to nearly absent, appearing like the shorter, ventricose cheilocystidial elements. ***Cheilocystidia:*** (18.2) 24.6–31.0–39.0 (41.9) × (6.2) 6.4–8.2–9.9 (13.2) µm, N = 20, hyaline, thin-walled but sometimes with a gradually thickening wall toward apices, variable in shape, and sometimes long and thin with 2-3 septa similar to the pleurocystidia found in *Pluteus septocystidiatus*, typically ventricose rostrate to lageniform to subampullaceous, occasionally ovate to ventricose mucronate, apical protrusion absent, short, rarely constricted or subcapitate. ***Subhymenium:*** a narrow zone of shorter, tortuous, branching, somewhat tightly packed elements. ***Hymenophoral trama:*** composed of parallel/subparallel, hyaline, thin-walled, mostly barrel-shaped cells. Faintly pigmented in masse. ***Caulocystidia***: Abundant near apex, absent below. 13.2–63.8 × 5.5–17.5 µm, highly variable in shape and arrangement, often chaining or with subtending cells, sometimes chaining perpendicular to stipitipellis while at other times chaining parallel with perpendicular cystidial protrusions, often fusiform ventricose, ampullaceous to lageniform, less commonly obpyriform to tapering from a wide base, rarely clavate to flexuous cylindrical, nearly always inequilateral, apices typically with a long, tapering, flexuous to constricted protrusion, less often mucronate, rarely obtuse, subcapitate, or rounded.

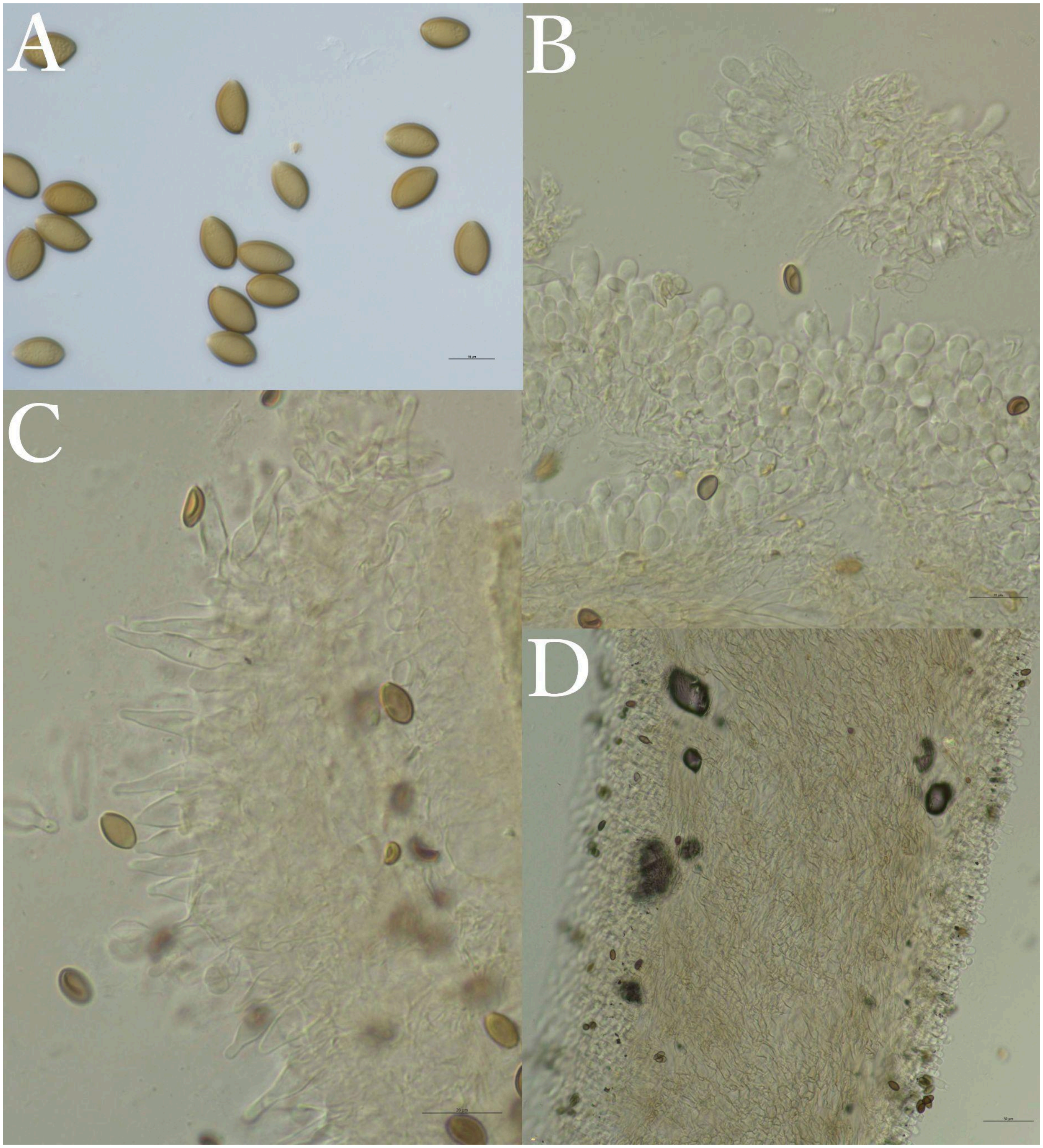

Figure 4. Microscopic characters of holotype collection mounted in glycerol. A) Basidiospores. B) Basidia and Basidioles C) Cheilocystidia. D) Lamellar trama. (Micrographs: Scott Ostuni and Andrew Kunik)

Figure 5. Basidiospores mounted in KOH at 1000x magnification (Micrograph by Alan Rockefeller)

Pileipellis: A discrete cutis of hyaline, more-or-less parallel to loosely interwoven, repent elements forming a distinct layer approximately 125–175 µm. Individual elements 3.5–6.0 µm wide, with undifferentiated terminal elements, apices rounded to obtuse. Individual elements sometimes collapsing with age, especially near the center of the pileus creating a pseudoixocutis. Over a narrow, pigmented, relatively discrete hypodermium of densely packed, medium orange-brown, interwoven elements. ***Pileus trama:*** Composed of frequently branched, cylindrical to oblong fusiform, hyaline, loosely interwoven elements 5.5–19.8 µm broad with slightly thick walls. Becoming more tightly packed and more parallel toward the hymenium. ***Stipe trama:*** Composed of parallel to subinterwoven, cylindrical, pigmented in masse, slightly thick-walled elements up to 20 µm broad. Broader elements are more parallel and shorter while narrower elements are more interwoven and longer. **Stipitipellis:** Composed of a narrow band of parallel, undifferentiated elements approximately 2-3 µm broad. ***Clamp connections*** present in all tissues.

Comments

This species is recovered in a clade with *P. aztecorum* and *P. serbica* (and its varieties), with high support (Figure 3). While this species shares similar micromorphological characters (i.e., ellipsoid to subellipsoid spores, cystidial distribution and shape) as well as an evanescent, subcortinate partial veil that does not leave an annulus, it can be differentiated by its broader stature (“collyboid” as opposed to “mycenoid” with a lower height-width ratio) along with spore dimensions. Other, macroscopically similar species (e.g., *P. ovoideocystidiata*) can be differentiated by the combination of the nearly complete absence of velar remnants in maturity and the broader stature.

This clade (along with the *P. cyanescens* clade) has been referred to as clade II-C (Ramírez-Cruz et al., 2013) but subclade C is not recovered as monophyletic, consistent with the genomic phylogeny produced by Bradshaw et al. (2024). The portion of clade II-C containing *P. caeruleorhiza* corresponds to *Psilocybe* section *Aztecorum* Guzmán while section *Cyanescens* is available for the other clade centered around *P. cyanescens*. Given the consistent recovery of major clades across studies, an updated, natural infrageneric classification should be proposed as no previous subgeneric or sectional classification systems correlates well with recovered evolutionary relationships.

It has been suggested that this species could have been imported into the region on commercially distributed wood-chips, raising questions concerning the geographic origin of this species. It is also possible that the species is native to the region and has been able to flourish in artificial wood-chip habitats. Given the ability to grow in colder climates and winter fruiting, if the species is introduced, it is likely from a temperate climate. This species may have been previously overlooked as it can strikingly resemble an *Agrocybe*, also commonly encountered in the same habitats, but can be readily distinguished by the blue-green bruising.

DISCUSSION

The discovery of this formerly undescribed species is surprising given the attention that the genus *Psilocybe* receives, yet it represents the second species in the genus described from the United States this year (Ostuni et al., 2024). These findings highlight the importance of community scientists’ contributions to databases like iNaturalist and Mushroom Observer as well as high-throughput DNA barcoding initiatives lead by amateur mycologists such as Ohio Mushroom DNA Lab, Mycota Labs, and FUNDIS (Cantonwine et al., 2022; Russell, 2022; Canan et al., 2024). If the mycological and conservation community hope to fully understand the biodiversity of fungi in North America, it is essential that community driven efforts continue to grow at an exponential pace while building bridges between the academic and citizen science community to close the taxonomic impediment (Cao et al., 2016; Canan et al., 2024. There has been a marked increase in social and cultural interest in mushrooms (psilocybin containing and otherwise) over the past several years due to increased education and awareness of their functional, therapeutic, and medicinal uses. The ever-shifting attitude towards fungi is likely to continue, ushering in new waves of bright-eyed mycophiles and myconauts alike for years to come. The authors hope that the discovery of *P. caeruleorhiza* (among other recently described species) stimulates more interest in the field of mycology and specifically fungal taxonomy and organismal biology.

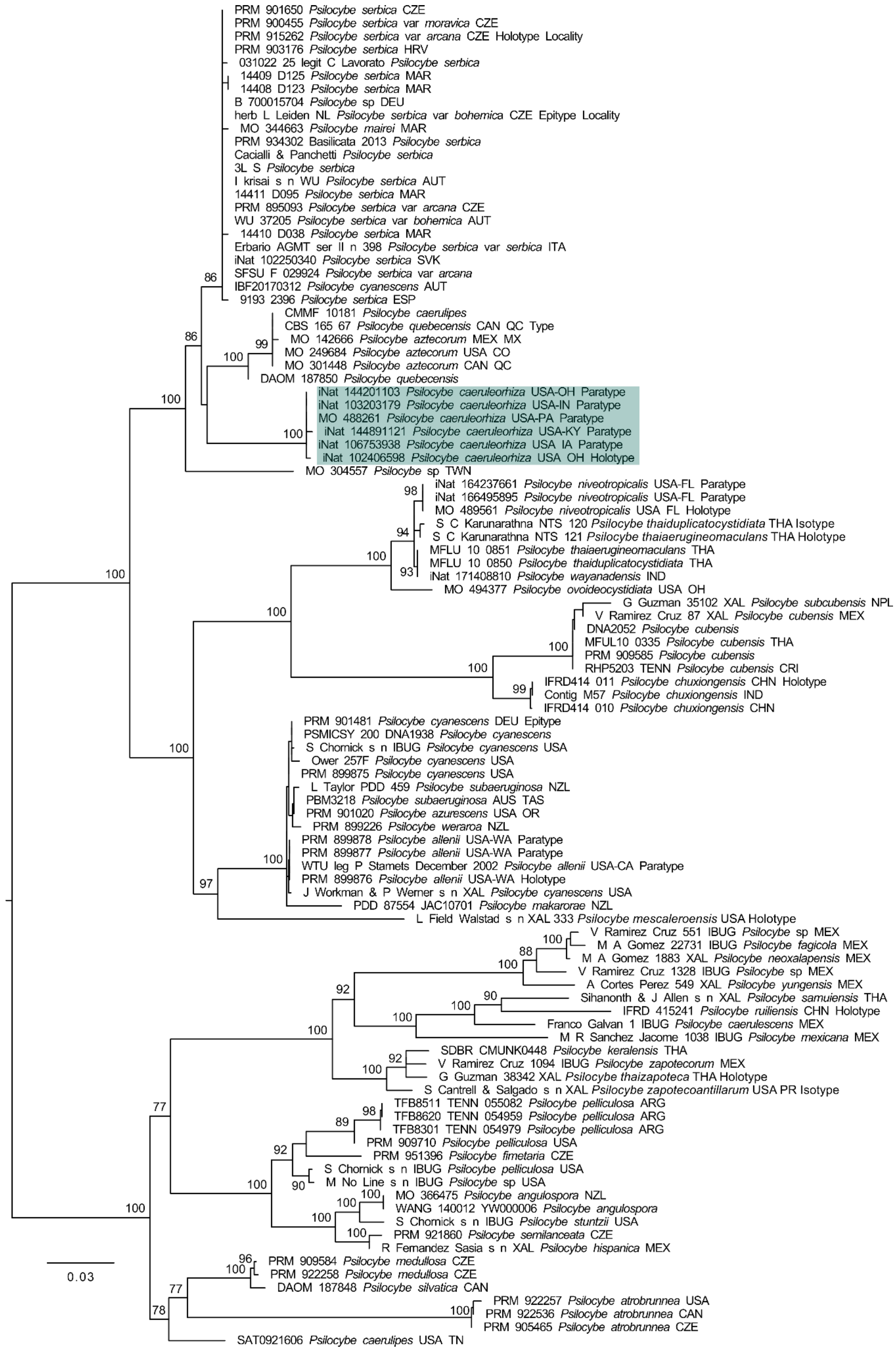

Figure 3. Maximum likelihood phylogenetic reconstruction based based on nrITS, nrLSU, rpb1 (intron and exon), rpb2 (exon), and tef1 (intron and exon) with *Psilocybe caeruleorhiza* highlighted in teal. Transfer bootstrap values >75 given at the branch nodes.

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SUPPLEMENTARY FILES

Supplementary File 1 - Sequences Used
https://docs.google.com/spreadsheets/d/1Rr56JHhrgMDlM-TQWInAbSogOg85BshCarhNq5P7tv4/edit?gid=0#

Supplementary File 2 - Alignment
https://drive.google.com/file/d/121lfpFNiDmSiW4IBqHQt-Zim8R-xYF6C/view?usp=sharing

Supplementary File 3 - Partition and Nucleotide Substitution Models
https://drive.google.com/file/d/1TVzu6O5R1Co6_nUoFoITHYJl7-BuVF_a/view?usp=sharing

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