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Taxonomy and systematics

Cyanoboletus abieticola (Boletaceae, Basidiomycota), a new species from Mexico

Cyanoboletus abieticola *(Boletaceae, Basidiomycota), una especie nueva de México*

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Abstract

Cyanoboletus abieticola is described as a new species to science. This species is characterized by the viscid pileus, cylindric to bacilliform basidiospores ([14.1] 16.2 ± 1.1 [17.5] × [5] 5.5 ± 0.3 [5.9] µm, Q [2.8] 3.0 ± 0.1 [3.2]), and its gregarious habit in mixed coniferous forests dominated by *Abies religiosa* or *A. guatemalensis*. Photographs, drawings, and the phylogenetic analysis of 3 genetic data sets (ITS, nucLSU, and RPB2) of the new species are presented.

Keywords: Boletales; Mycorrhizal fungi; "*Pulveroboletus* group"; *Abies*

Resumen

Cyanoboletus abieticola es descrita como una especie nueva para la ciencia. Esta especie se caracteriza por el píleo víscido, basidiosporas cilíndricas a baciliformes ([14.1] 16.2 ± 1.1 [17.5] × [5] 5.5 ± 0.3 [5.9] µm, Q [2.8] 3.0 ± 0.1 [3.2]) y su hábito gregario en bosques mixtos de coníferas dominados por *Abies religiosa* o *A. guatemalensis*.

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Se presentan fotografías, dibujos y el análisis filogenético de 3 conjuntos de datos genéticos (ITS, nucLSU y RPB2) de la nueva especie.

Palabras clave: Boletales; Hongos ectomicorrizógenos; "Grupo *Pulveroboletus*"; *Abies*

Introduction

The family Boletaceae comprises pileate-stipitate or sequestrate species, with poroid or lamellate hymenophore. Boletaceae species are an important component of ecosystems because most of them form ectomycorrhizal associations with a great diversity of angiosperms and gymnosperms (Binder & Hibbet, 2006; Ortíz-Santana et al., 2007). They are common in different vegetation types, such as deciduous, conifer, and mixed forest; mediterranean shrublands, tropical and montane cloud forests (Bessette et al., 2010; Gelardi, 2020; Leonardi et al., 2020; Wu et al., 2016). In addition to their ecological importance, many species are valued edibles in several regions of the world (Garibay-Orijel et al., 2009; Gelardi, 2020).

Wu et al. (2014) recognized 7 major clades at the subfamily level and 59 genus-level clades, placing *Boletus pulverulentus* within the "*Pulveroboletus* group", which also includes the genera *Butyriboletus* D. Arora & J.L. Frank, *Cacaoporus* Raspé & Vadthanarat, *Caloboletus* Vizzini, *Crocinoboletus* N.K. Zeng, Zhu L. Yang & G. Wu, *Gymnogaster* J.W. Cribb, *Lanmaoa* G. Wu & Zhu L. Yang, *Pulveroboletus* Murrill, *Rubroboletus* Kuan, *Rugiboletus* G. Wu & Zhu L. Yang, *Suillellus* Murrill, and *Sutorius* Halling, Nuhn & N.A. Fechner. Members of the "*Pulveroboletus* group" share certain common characteristics such as poroid hymenium, usually yellowish, reddish or brown hymenophore, *Boletus*-type hymenophoral trama (Snell & Dick, 1958), and smooth spores.

Gelardi et al. (2014) proposed the new genus *Cyanoboletus*, within the "*Pulveroboletus* group", for those species characterized mainly by their strong blue reaction of basidiomata and context to cutting. They included 3 species in this genus: *C. pulverulentus* (Opat.) Gelardi, Vizzini & Simonini, *C. sinopulverulentus* (Gelardi & Vizzini) Gelardi, Vizzini & Simonini, and *C. rainisiae* (Bessette & O.K. Mill.) Gelardi, Vizzini & Simonini. The latter one was transferred to the genus *Xerocomellus*, but without type studies, and is currently known as *X. rainisiae* (Bessette & O.K. Mill.) N. Siegel, C.F. Schwarz & J.L. Frank (see discussion in Frank et al. [2020]).

Later, 4 more species were transferred to this genus: *C. cyaneitinctus* (Murrill) A. Farid, A.R. Franck & J.A. Bolin (Farid et al., 2021), *C. instabilis* (W. F. Chiu) G. Wu & Zhu L. Yang (Wu et al., 2016), *C. poikilochromus* (Pöder, Cetto & Zuccherelli) M. Carbone, D. Puddu & P. Alvarado (Carbone et al., 2023), and *C. flavosanguineus* (Lavorato & Simonini) Pierotti (Pierotti, 2015). However, the latter species was placed in the genus *Neoboletus* in 2021: *N. flavosanguineus* (Lavorato & Simonini) Biketova, Wasser, Simonini & Gelardi (Biketova et al., 2021).

Additionally, 5 *Cyanoboletus* species have been recently described: *C. brunneoruber* G. Wu & Zhu L. Yang from China (Wu et al., 2016), *C. hymenoglutinosus* D. Chakr., K. Das, A. Baghela, S.K. Singh & Dentinger nom. inval. from India (Li et al., 2016), *C. bessettei* A.R. Bessette, L.V. Kudzma, & A. Farid from the USA (Farid et al., 2021), *C. macroporus* Sarwar, Naseer & Khalid from Pakistan (Sarwar et al., 2021), and *C. mediterraneensis* Biketova, A. Rinaldi & Simonini from Israel and Italy (Biketova et al., 2016, 2022). Therefore, 10 species are currently known for the genus. These species are distributed mainly in Asia, Europe and North America and associated with *Abies*, *Arbutus*, *Buxus*, *Carya*, *Castanopsis*, *Cistus*, *Crataegus*, *Cryptomeria*, *Erica*, *Juniperus*, *Lithocarpus*, *Ostrya*, *Pinus*, *Pistacea*, and *Quercus* trees, as well as with *Halimium* shrub (Biketova et al., 2022; Farid et al., 2021; García et al., 1998; Li et al., 2016; Wu et al., 2016).

This paper describes *Cyanoboletus abieticola* as a new species from Mexico associated with mixed conifer forests dominated by *Abies religiosa* (Kunth) Schltdl. & Cham. and *A. guatemalensis* Rehder.

Materials and methods

Collections were carried out in central and southern Mexico (Fig. 1), in mixed coniferous forests, during the rainy season from June to October (2000-2017). Morphological characteristics were described according to Largent (1977) and Lodge et al. (2004). Chemical reactions with KOH 5% and Melzer reagent were tested on pileus, hymenophore, and stipe surface. Photographs of basidiomata were taken on site, as well as data regarding plant species. The colors for the taxonomic description were based on Kornerup and Wanscher (1978). A light microscope (Carl Zeiss GmbH 37081, Germany) was used to observe the microscopic characteristics. Only structures from mature basidiomes were measured. Twenty basidiospores, basidia, pleurocystidia, cheilocystidia and caulocystidia were measured by specimen. If the specimen consisted of several basidiomes, only the structures of 2 of

them were measured. The mean of each variable of every collection was computed. Dimensions are given according to the following format: (lower mean) mean of means ± standard deviation (upper mean), Q = (lower mean) mean of means ± standard deviation (upper mean).

Vouchers were deposited in the "Herbario Nacional de México" (MEXU), in the José Castillo Tovar herbarium (ITCV), and in the mycological herbarium of the Universidad Autónoma de Querétaro (MUAQ). Additional materials were obtained in loan from the following herbaria: Escuela Nacional de Ciencias Biológicas-Instituto Politécnico Nacional herbaria (ENCB-IPN), Instituto de Biología Universidad de Guadalajara (IBUG), Instituto Nacional de Ecología (INECOL) and Universidad Autónoma de Nuevo León (UNL). All acronyms of herbaria and mycological collections follow Index Herbariorum (Thiers, 2024).

Samples of dehydrated basidiomata were used for DNA extraction. The DNA was extracted using the DNeasy Power-Soil kit (QIAGEN). Cell lysis was performed by grinding in a mortar with liquid nitrogen. Three nuclear loci (ITS, LSU and RPB2) were amplified with Platinum Taq DNA Polymerase (Invitrogen-Thermo Fisher Scientific) or Taq & Load PCR Mastermix (MP Biomedicals) in a thermal cycler (BIO-RAD). The PCR parameters were as follows: 95 ºC initial denaturation 4 min; 35 cycles of denaturation at 94 ºC for 1 min, annealing at 54 ºC for 1 min, extension at 72 ºC for 1 min, and a final extension step at 72 ºC for 10 min. The primers ITS1/ITS4 (White et al., 1990) were used for the ITS region; LR0R/LR5 (Vilgalys & Hester, 1990) for nucLSU; and RPB2-B-F2/RPB2-B-R (Wu et al., 2014) for the RPB2 gene. The PCR products were examined by 1% agarose gel electrophoresis, the gel was stained with GelRed (Biotium) and observed under an UVP Multidoc-It transilluminator (Analytikjena). Successful PCR products were cleaned with diluted 1:1 ddH2O: ExoSAP-IT (Thermo Fisher Scientific) and incubated at 37 ºC for 45 min and 80 ºC for 15 min. Sanger sequencing of clean PCR products was performed in the "Laboratorio de secuenciación genómica de la biodiversidad y la salud" at Instituto de Biología, Universidad Nacional Autónoma de México. Samples were sequenced in both directions with PCR primers using BigDye Terminator v3.1 (Thermo Fisher Scientific). Sequences were edited in Chromas Pro Vers. 1.41 (Technelysium Pty, Ltd, Tewantin, Qld, Australia).

For phylogenetic analyses we used *Cyanoboletus* sequences from the ITS, nucLSU and RPB2 regions listed in the Table 1. A total of 32 concatenated sequences were used (Supplementary material 1). Two *Lanmaoa* species were chosen as an outgroup. The sequences of each locus were aligned separately using MacClade 4.0 (Maddison & Maddison, 2000) and concatenated in Mesquite 3.40 (Maddison & Maddison, 2018). The alignments were manually edited to eliminate ambiguous regions.

Phylogenetic analyses were partitioned for both Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. Best evolutionary models were selected with jModelTest 2.1.10 (Darriba et al., 2012), using the Akaike information criteria. Table 2 summarizes the evolutionary model of each region. The ML analysis was performed in RAxML 7.2.6 (Stamatakis, 2006), with 1,000 bootstrap replicates. The BI analysis was performed in MrBayes 3.2.5 (Ronquist & Huelsenbeck, 2003) with 5,000,000 generations, 4 Monte Carlo chains and sampling every

Table 1
List of DNA sequences used for *Cyanoboletus* phylogenetic analyses.

Species	Code	Country	GenBank			Reference
			ITS	nucLSU	RPB2	
Lanmaoa sublurida	USF 288426	USA	MW675740	MW662575	MW737499	Farid et al., 2021
L. sublurida	USF 300104	USA	MW675736	MW662572	MW737498	Farid et al., 2021
Cyanoboletus abieticola	MUAQ13 Paratype	Mexico	MW750332	MW750369	-	This study
C. abieticola	ITCV-1010 Paratype	Mexico	-	MW750367	-	This study
C. abieticola	MEXU-30111 Paratype	Mexico	MW209739	MW750366	PP108649	This study
C. abieticola	MEXU-30106 Paratype	Mexico	MW209740	MW750365	-	This study
C. abieticola	MEXU-30109 Holotype	Mexico	MW209738	MW750368	PP108650	This study

Table 1. Continued

Species	Code	Country	GenBank			Reference
			ITS	nucLSU	RPB2	
C. abieticola	MEXU-26275 Paratype	Mexico	KC152077	-	-	This study
C. abieticola	MEXU-26276 Paratype	Mexico	KC152076	-	-	This study
C. abieticola	MEXU-26278 Paratype	Mexico	KC152075	-	-	This study
C. bessettei	USF 301500 (A) Holotype	USA	MW675737	MW662571	MW737457	Farid et al., 2021
C. bessettei	USF 301500 (B) Holotype	USA	MW675738	-	MW737458	Farid et al., 2021
C. brunneoruber	HKAS63504	China	-	KF112368	KF112702	Wu et al., 2014
C. brunneoruber	HKAS80579 1	China	-	KT990568	KT990401	Wu et al., 2016
C. brunneoruber	HKAS80579 2	China	-	KT990569	KT990402	Wu et al., 2016
C. cyaneitinctus	USF 288424	USA	MW675739	MW662574	MW737461	Farid et al., 2021
C. cyaneitinctus	USF 301499 Epitype	USA	MW675744	MW662579	MW737503	Farid et al., 2021
C. cyaneitinctus	JAB184	USA	MW675731	MW662584	MW737467	Farid et al., 2021
C. cyaneitinctus	JAB324	USA	MW675732	MW662586	MW737469	Farid et al., 2021
C. cyaneitinctus	JAB325	USA	MW675733	-	MW737470	Farid et al., 2021
C. hymenoglutinosus	AB2016	India	KT907355	KT860060	-	Li et al., 2016
C. instabilis	FHMU1839	China	MG030473	MG030466	-	Chai et al., 2018
C. instabilis	HKAS59554	China	-	KF112412	KF112698	Wu et al., 2014
C. macroporus	DC21-02	India	OQ860238	OQ860239	ON364552	Das et al., 2023
C. macroporus	DC21-04	India	OQ860240	OQ860241	OQ876894	Das et al., 2023
C. mediterraneensis	K-M000265123 Holotype	Israel	-	OM801212	-	Biketova et al., 2023
C. mediterraneensis	K-M000265124 Paratype	Israel	OM801199	-	-	Biketova et al., 2023
C. mediterraneensis	TUR-A 209199	Italy	MZ265183	MZ265198	MZ277228	Carbone et al., unpublished
C. paurianus	KD22-008	India	-	OQ859920	OQ914389	Das et al., 2023
C. paurianus	KD22-009 Holotype	India	-	OQ859919	OQ914388	Das et al., 2023
C. poikilochromus	GS10070	Italy	KT157051	KT157060	KT157068	Gelardi et al., 2015
C. poikilochromus	GS11008	Italy	KT157050	KT157059	KT157067	Gelardi et al., 2015
C. poikilochromus	TO HG 100091987 Epitype	Italy	KT157047	KT157056	-	Gelardi et al., 2015
C. pulverulentus	MG126a	Italy	KT157053	KT157062	-	Gelardi et al., 2015
C. pulverulentus	MG 456a	Portugal	KT157054	KT157063	-	Gelardi et al., 2015
C. pulverulentus	MG 628a	Italy	KT157055	KT157064	KY157069	Gelardi et al., 2015
C. sinopulverulentus	HKAS59609	China	-	KF112366	KF112700	Wu et al., 2014
C. sinopulverulentus	HMAS266894	China	KC579402	-	-	Gelardi et al., 2015
Cyanoboletus sp.	HKAS76850	China	-	KF112343	KF112697	Wu et al., 2014

Table 2
Nucleotide substitutions models by each partition obtained from jModelTest with the corrected Akaike information criterion.

Gene/Partition	Length	Evolutifon model	Nst-rates
nucLSU	747 pb	TIM1+G	6 – Gamma
ITS	603 pb	TPM3ef+G	6 – Gamma
RPB2	688 pb	TIM3ef+G	6 – Gamma

100 trees, chain convergence was determined using Tracer 1.7.2 (Rambaut et al., 2018), we discarded the first 25% of trees as burning. ML bootstrap support (BS) over 50% and Bayesian posterior probability (PP) values exceeding 0.90 are reported in the resulting tree.

Results

The phylogenetic analyses (Fig. 1) based on the ITS, nucLSU and RPB2 concatenated dataset using ML analyses and BI showed that all *Cyanoboletus* species cluster in a monophyletic clade with strong support (PP 1 / BS 100). Trees obtained from separate ITS and RPB2 analyses are presented in Supplementary material 2. Sequences of all *C. abieticola* sp. nov. samples form a strongly supported clade (PP 1/BS 99) within the genus *Cyanoboletus*. Consequently, based on morphological, phylogenetic and ecological data, we describe it as a new species, which is also the first species of the genus *Cyanoboletus* reported from Mexico.

To determine whether the ITS or RPB2 region is more informative as a barcode, in Table 3 we show the percentages of nucleotide similarity between species. The RPB2 region commonly has more variation and generates fewer ambiguous regions than the ITS. Even while the ITS region has been considered as the fungal genetic barcode, the RPB2 region has higher resolution for species delineation in *Cyanoboletus*.

Description

Cyanoboletus abieticola J. García, Ayala-Vásquez & Landeros, sp. nov.

Diagnosis. Pileus 13-50 mm in diameter, viscid, widely convex, convex to plane convex, brown, yellow, pale brown, reddish-brown, cinnamon. Stipe 40-75 × 7-8 mm, viscid, cylindrical, yellow when young, middle section and apex yellow when mature, basal area red brown to red-vinaceous, and basidiospores (14.1) 16.2 ± 1.1 (17.5) × (5) 5.5 ± 0.3 (5.9) µm. It grows on the ground of neotropical *Abies* forests.

Description. Macroscopic characters (Fig. 2). Pileus 13-50 mm diameter, widely convex, convex to plane convex, brown yellow (4B6), pale brown (6B5), reddish-brown, cinnamon, furfuraceous, very viscid when young, incurved margin, sterile. Hymenophore attached, pores 0.3-0.7 mm in diameter, pale yellow (2A8-2A4), yellow (3A8) to yellow-olive (30B8-30B7) it stains dark blue (22F8) when touched, with some brown tones, young specimens exude a somewhat acidic astringent-flavored yellow liquid from hymenophore; tubes 2-6 mm diameter, concolor to pores, immediately changing to dark blue (25F8) when cut. Context white, 5 mm thick, dark blue (22F8) when cut; stipe context pale yellow (3A6) base red-vinaceous (10F8), turning blue (25F8) when cut. Stipe 40-75 × 7-8 mm, cylindrical, yellow when young, middle section and apex yellow when mature, basal area red brown (9C8-9C5) to red-vinaceous (10F8), surface pruinose to furfuraceous, immediately turning dark blue (22F8) when touched. Mycelium white.

Chemical reactions: pileus surface and context turning dark brown (6F8) with KOH 5%, hymenophore turning brown (5F3) with KOH 5%.

Microscopic characters (Fig. 3). Basidiospores 14.1-17.5 × 5-5.9 µm, mean values 16.2 ± 1.1 × 5.5 ± 0.3 µm, Q 2.8-3.2, means values 3.0 ± 0.1, cylindric to bacilliform, yellow in KOH, inamyloid with Melzer's reagent, with visible suprahilar depression (Fig. 3A). Basidia 27.3-37.6 × 9.5-10.7 µm, mean values 33.3 ± 4.0 × 10.3 ± 0.5 µm, clavate, hyaline in KOH, tetrasporic (Fig. 3B). Hymenophoral trama divergent (*Boletus*-type), with a medium and lateral stratum of cylindrical hyphae, hyaline to yellowish brown in KOH, inamyloid with Melzer's reagent, with gelatinized wall. Pleurocystidia 52.2-71.2 × 10.6-13.9 µm, mean values 63.2 ± 7.5 × 12.4 ± 1.4 µm, arise from subhymenium, mucronate, clavate, fusoid-ventricose, hyaline to brown in KOH (Fig. 3C), with reddish brown incrustations on Melzer. Cheilocystidia 39.6-58.4 × 8.9-11 µm, mean values 47.7 ± 7.5 × 9.9 ± 0.8 µm, fusoid-ventricose, mucronate, clavate, reddish to brown on KOH, with reddish brown incrustations (dextrinoid) in Melzer's reagent, thick-walled (Fig. 3D). Pileipellis formed by an ixotrichoderm 250-300 µm thick, with terminal cells 34.8-45.4 × 4-5.6 µm, mean values 38.4 ± 4.8 × 4.7 ± 0.6 µm, cylindrical, yellow-reddish brown in KOH, reddish brown (dextrinoid) with Melzer's reagent, some with thick wall, sometimes gelatinized (Fig. 3E). Stipitipellis 100-120 µm thick, ixocutis, hyphae subparallel to loosely intermingled, formed of caulocystidia 34-47 × 9.6-11.4 µm, mean values 40.6 ± 5.2 × 10.3 ± 0.7 µm, in clusters, fusoid-mucronate, clavate, some ventricose, arise from the middle or surface, hyaline to brown in KOH, with reddish brown incrustations (dextrinoid) in Melzer's reagent.

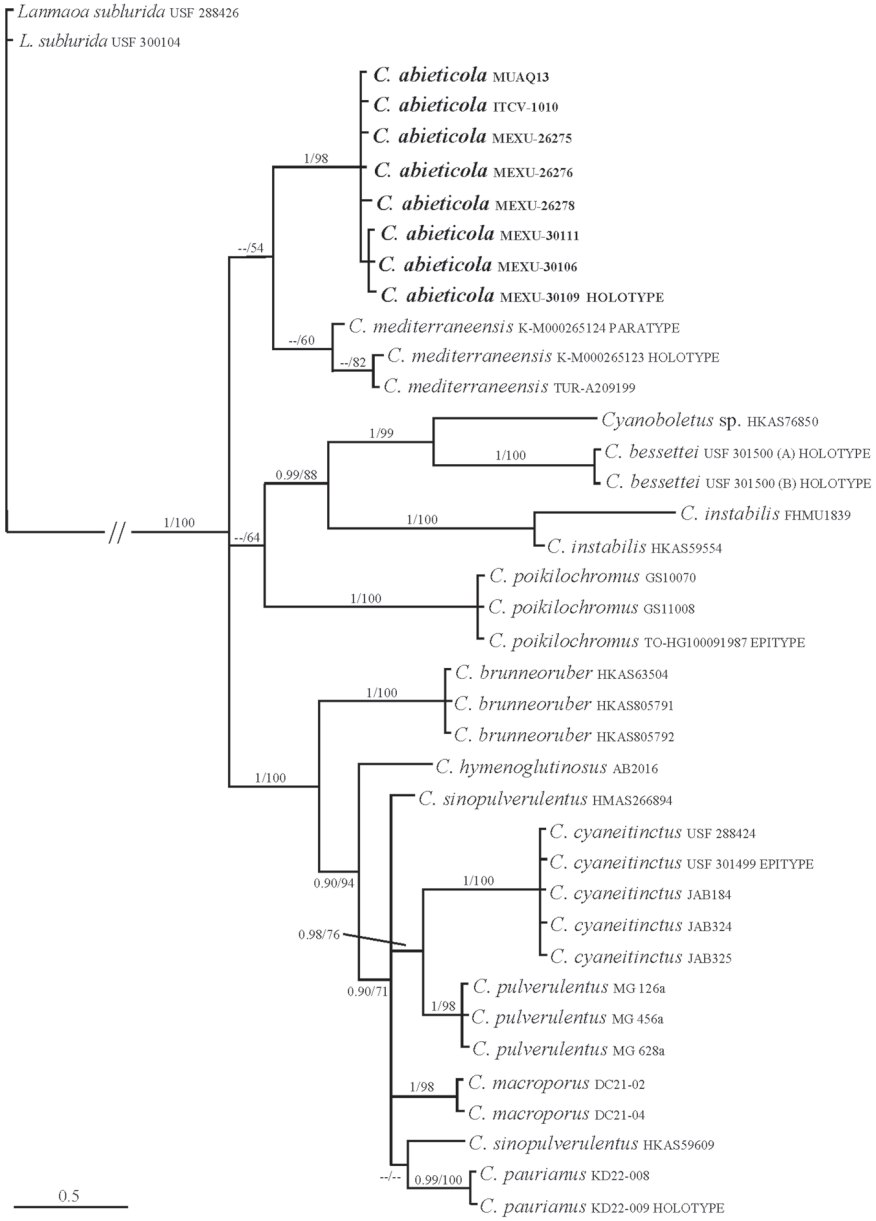

Figure 1. Bayesian tree using the concatenated alignment (LSU, ITS and RPB2). The values in the phylogram branches represent the maximum likelihood bootstrap (MLB)/Bayesian posterior probabilities (BPP). Only MLB 50 ≥ and 90 BPP ≥ are displayed. *Cyanoboletus abieticola* is in bold.

Taxonomic summary

Holotype: Mexico, Oaxaca, Santa Catarina Ixtepeji, La Cumbre, *Abies guatemalensis*, 17º11' N, 96º38' W, 2,902 m asl, 4 November 2017, Ayala-Vásquez (MEXU-30109).

Isotype: ITCV-1141

Mycobank: MB 838523.

Etymology: the name of the species is due to its association with *Abies* forests.

Habitat: scattered or solitary in mixed neotropical conifer forests dominated by *Abies religiosa* and *A. guatemalensis*.

Additional material studied. Mexico, Hidalgo: El Chico National Park, *Abies religiosa*, 13 August 1988, J. García (ITCV-5874), 13 August 1988, J. García (ITCV-5878), 23 August 2018, J. García (ITCV-21996); Jalisco: Road to Tamazula, 15 km to Mazamitla, between Guayabos

Table 3

Percent nucleotide similarity between *Cyanoboletus* species based on their sequences, ITS/RPB2 DNA sequences. Above ITS and below RPB2 percentage ranges (WD: without data).

	C. abieticola	*C. bessettei*	*C. brunneoruber*	*C. cyaneitinctus*	*C. hymenoglutinosusus*	*C. instabilis*	*C. macroporus*	*C. mediterraneensis*	*C. paurianus*	*C. poikilochromus*	*C. pulverulentus*	*C. sinopulverulentus*
C. abieticola	0.0-0.5 0.0											
C. bessettei	5.6-6.2 8.7	0.0 0.0										
C. brunneoruber	WD 5.9	WD 8.7	WD 0.0									
C. cyaneitinctus	6.7-7.2 7.0	8.1 10.2	WD 5.9	0.0 0.0								
C. hymenoglutinosus	3.6-3.9 WD	4.6 WD	WD WD	5.7 WD	WD WD							
C. instabilis	5.7-7.0 7.5	5.7 8.3	WD 7.8	6.3 8.7	6.3 WD	WD WD						
C. macroporus	4.4-4.6 6.0	5.4 8.8	WD 5.3	4.9 3.6	1.8 WD	7.0 7.9	0.0 0.0					
C. mediterraneensis	2.9-3.9 2.7	2.9-3.4 7.0	WD 5.4	2.0-5.9 6.0	2.3 WD	5.1 5.8	3.1-3.2 5.4	0.0-0.3 WD				
C. paurianus	WD 6.2	WD 9.2	WD 4.6	WD 3.6	WD WD	WD 8.0	WD 2.7	WD 4.9	WD 0.0			
C. poikilochromus	4.2-5.9 6.9-7.1	3.9 8.5-8.7	WD 7.1-7.4	5.9-7.6 7.7-7.8	2.0-3.4 WD	7.0-7.1 7.8-8.0	3.3-4.6 7.5	1.7-3.0 6.3-6.7	WD 7.1-7.2	0.0 0.0-0.5		
C. pulverulentus	3.6-3.9 6.5	4.9-5.2 9.1	WD 5.2	4.6 2.2	2.1-2.3 WD	6.3 8.4	1.8-2.1 2.8	2.3-2.8 5.1	WD 2.8	3.6-4.9 7.8	0.0-0.3 WD	
C. sinopulverulentus	3.8-4.1 6.1	5.2 9.3	WD 4.6	4.0 3.9	2.8 WD	6.3 7.4	1.0 2.7	2.3-3.1 4.7	WD 2.1	3.1-4.7 7.0-7.1	1.3-1.6 3.3	WD WD

and Cabañas, *A. religiosa*, 24 August 1974, G. Guzmán (ENCB-11869); Road from San Sebastián del Oeste to Santa Ana, *A. religiosa*, 29 August 1994, L. Guzmán-Dávalos (IBUG-5349); Nevado de Colima, *A. religiosa*, 13 October 1984, A. Tamayo and R. González (IBUG); 11 August 1990, J. García (ITCV-6633); Estado de México: Road to Chalma, Lagunas de Zempoala National Park, *A. religiosa*, 1 August 1982, R. E. Chio (ENCB-368, ITCV-368), 23 September 1985, E. Perez-Silva (MEXU-19733), 17 July 1985, García (ITCV-4784); Nevado de Toluca National Park, near Ranchería La Puerta, *A. religiosa*, 21 August 1983, L. Colón 208-a (ENCB); Naucalpan-Toluca highway, road to Villa Alpina, La Glorieta, *A. religiosa*, without date, A. González-Velázquez (ENCB-965); Mpio. Amanalco, Corral de Piedras, *A. religiosa*, without date, A. González-Velázquez (ENCB-1454); Zone E of San Rafael Atlixco, Escualango, *A. religiosa*, 7 October 1983, A. Hernández (ENCB-145); La Marquesa, *A. religiosa*, 7 July 1963, Gispert-Imaz (MEXU-9873), 12 August 1962, G. Guzman (ENCB-3198); Ciudad de México: Former Mexico-Cuernavaca highway 3 km S. of Parres, *A. religiosa*, 17 July 1982, S. Chacón (ENCB-183); Michoacán: Morelia-Cd Road. Hidalgo, Sierra de Mil Cumbres, km 45, *A. religiosa*, 21 July 1983, J. García (UNL-3665, ITCV-3665), 15 July 1982, J. García (UNL-1998, ITCV-1998); Morelos: Road to Chalma, 5 km Huitzilac, *A. religiosa*, 17 July 1982, S. Chacón (ENCB-234); Oaxaca: Santa Catarina Ixtepeji, La Cumbre, 17°11' N, 96°38' W, *A. guatemalensis*, 18 July 2017, Ayala-Vásquez (ITCV-846), 20 August 2017, Ayala-Vásquez (ITCV-1002), 21 August 2017, Ayala-Vásquez and Victores-Aguirre, (ITCV-1010); 8 October 2017, Ayala-Vásquez, (MEXU-30106, ITCV-1129),

Figure 2. *Cyanoboletus abieticola* morphology. A) Basidiomata (holotype), B) context, C) basidiomata at different stages. Bar = 10 mm.

Ayala-Vásquez (MEXU-30111, ITCV-1128); 4 November 2017, Ayala-Vásquez (ITCV-1136); Ayala-Vásquez (MEXU-30109, ITCV-1141), Ayala-Vásquez, (ITCV-1142); Querétaro: Mpio. Cerro El Zamorano, *A. religiosa*, 16 September 1995, J. García (ITCV-9560), 23 September 2017, Ferrusca 390 (MUAQ13), 19 october 2019, Ferrusca 462 (MUAQ14), Ferrusca 467 (MUAQ15), 16 september 2022, Landeros 3770 (MUAQ16); Tlaxcala:

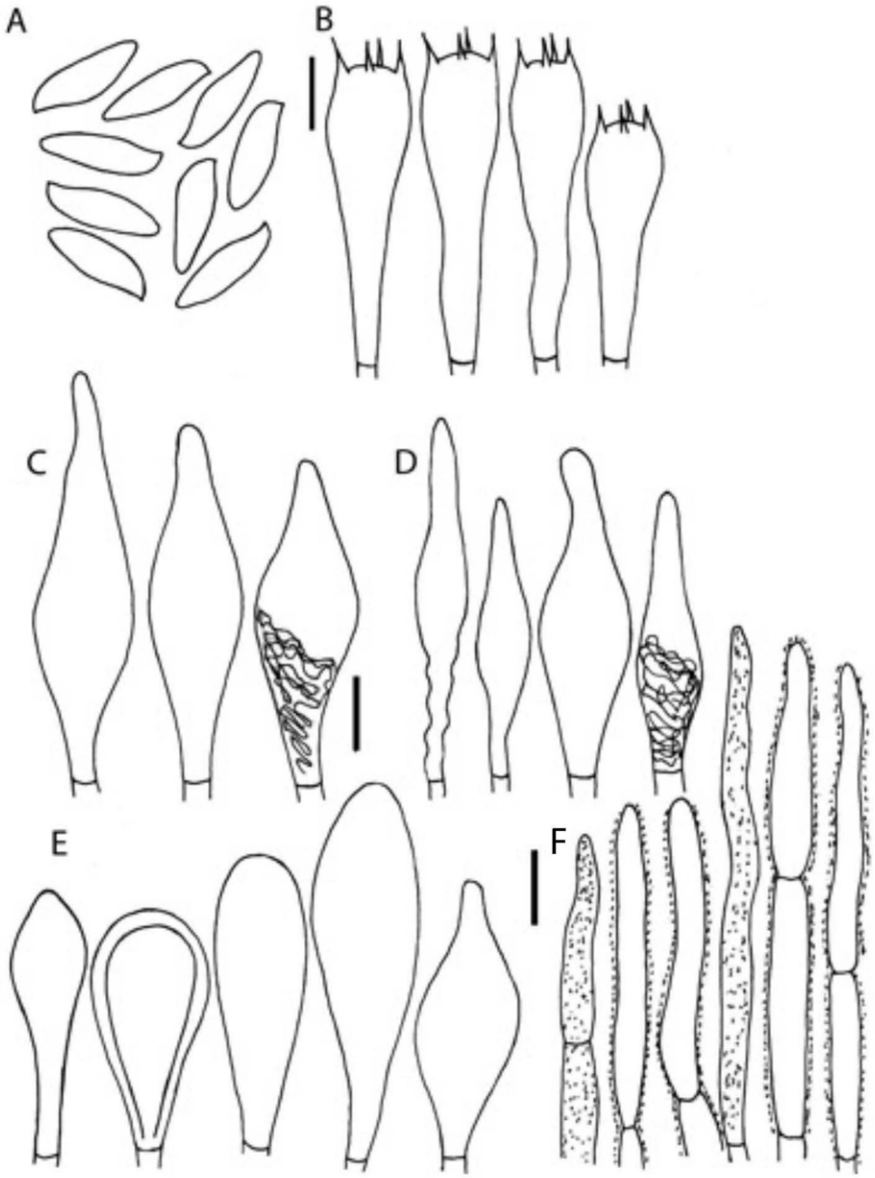

Figure 3. Microscopic characteristics of *Cyanoboletus abieticola*. A) Basidiospores, B) basidia, C) cheilocystidia, D) pleurocystidia, E) stipitipellis, F) pileipellis. Bar = 10 µm.

road from Tlaxco to Chignahuapan, Cerro de Teapa, El Conejo, *A. religiosa*, 26 June 1979, J. García (UNL-496, ITCV-496); Huamantla, road to La Malinche hilltop, *A. religiosa*, 29 August 2010, T. Bruns (MEXU-26275) (MEXU-26276) (MEXU-26278); Veracruz: Xico, Los Gallos, 1.5 km to N. from Ingenio El Rosario, Zona del Cofre de Perote, *A. religiosa*, 27 October 1983, Villarreal 1024 (INECOL-1068), 31 July 1990, J. García (ITCV-6459).

Remarks

Cyanoboletus abieticola is a new species with a strong phylogenetic support (PP1 / BS 99) and distinctive taxonomic characters. This species is characterized by a small pileus 13-50 mm diameter, very viscid, pale brown, yellowish-brown, brown, red to cinnamon; pileus sterile margin somewhat involute when young; hymenophore distills a liquid with sweaty acidic flavor when young, pores and tubes yellow; stipe yellow cylindrical in the

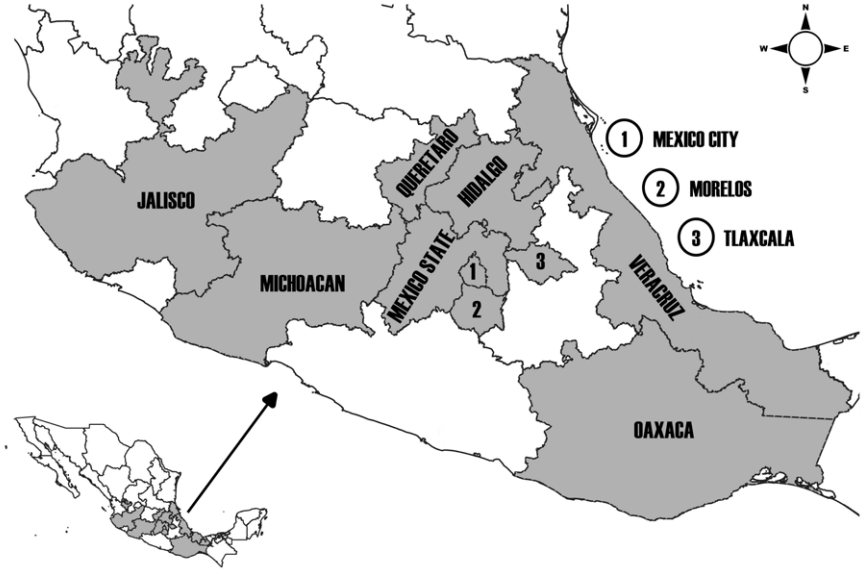

Figure 4. Distribution of *Cyanoboletus abieticola* in Mexico.

middle and apex, basal area red brown to red-vinaceous, when touched immediately turns to dark blue. It is distributed in mixed coniferous forests in central and southern Mexico at altitudes ranging from 2,700 to 3,000 m asl (Fig. 4). Previously, only 2 species of *Cyanoboletus* (*C. bessettei* and *C. cyaneitinctus*) have been recorded from North America (Farid et al., 2021). *Cyanoboletus bessettei* and *C. cyaneitinctus* have similar small basidiomata as *C. abieticola*, nevertheless, both species are associated with oak forests in the United States, in contrast, *C. abieticola* is distributed from central to southern Mexico in mixed conifer forests with a strong association with *Abies.* Morphological and microscopical comparisons of the American species of *Cyanoboletus* are shown in the Table 4. They are similar by morphology, however, *C. bessettei* has blue-green then reddish-brown staining in its stipe when handled, while *C. abieticola* and *C. cyaneitinctus* are bluing, also their basidiospores are bigger than those in *C. bessettei* (9-11 ´ 3.5-5 mm). *Cyanoboletus cyaneitinctus* and *C. abieticola* are very similar by morphology and microscopical characters, however they are not phylogenetically close, and the first one has shorter basidiospores in length (11.5-15 mm) and associates with *Carya* and *Quercus*.

Table 4

North American *Cyanoboletus* species morphology comparison.

Characteristic	*C. bessettei*	*C. cyaneitinctus*	*C. abieticola*
Pileus color	Buffy brown when young, darker brownish at maturity	Bister, umber, mahogany and dark brown	Brown yellow, pale brown, reddish brown, cinnamon
Pileus size	2.7-8 cm wide	3-8 cm wide	1.3-5 cm wide
Staining stipe when handled	Blue-green then reddish-brown	Bluing instantly and strongly	Bluing instantly and strongly
Stipe size	2.5-4 × 1-2 cm	3-6 × 0.5-2 cm	4-7.5 × 0.7-0.8 cm
Basidia	(2)4-spored	4-spored	4-spored
Basidiospores	(8-)9-11(-12) × 3.5-5 mm	(11)11.5-15(16) × 4-6 mm	[(14.1) 16.2 ± 1.1 (17.5) × (5) 5.5 ± 0.3 (5.9) µm
Host plants	Under *Quercus* and *Pinus*	Under *Carya* and *Quercus*	Under *Abies* spp.

Most *Cyanoboletus* species are morphologically similar, so to separate them, a combination of microscopic, geographic and molecular characteristics is necessary. With the description of this new species, the genus *Cyanoboletus* reaches its southernmost distribution in America, developing in forests of central and southern Mexico forests. Its occurrence in *Abies* forests is also highlighted, since in North America the genus has been recorded in mixed *Pinus-Quercus* forests (Farid et al., 2021).

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