



A contribution to the taxonomy and phylogeny of the genus *Pisolithus* (Sclerodermataceae, Boletales) in Mexico

Contribución a la taxonomía y filogenia del género *Pisolithus* (Sclerodermataceae, Boletales) en México

Iván Sandoval-Islas¹ and Eduardo Hernández Navarro^{1,2}

Abstract:

Background and Aims: The genus *Pisolithus* is characterized by its angiocarpic basidiomes, which have a pulverulent gleba composed of peridioles, apical maturation, and echinulated basidiospores. In Mexico, two closely related species have been cited based exclusively on morphology: *Pisolithus tinctorius* and *P. arhizus*, associated with *Pinus*, *Quercus*, *Carya* and *Eucalyptus*. However, it has been pointed out that none of those species form associations with eucalyptus, and *P. arhizus* is distributed just in Eurasia based on ITS sequences. This study aims to contribute to the taxonomy and phylogeny of the genus *Pisolithus* in Mexico by characterizing *Pisolithus* specimens from temperate and arid zones, based on morphology and ITS barcode sequences of fungarium specimens, as well as soil metabarcoding sequences.

Methods: Thirty-two specimens from the MEXU herbarium were characterized macro- and microscopically by light and scanning electron microscopy. Specialized literature was used for morphological identification. DNA was extracted with a CTAB 3% protocol. The ITS region was amplified with the ITS5 and ITS4 primer pairs. Phylogenetic analyses included 21 newly generated sequences, metagenomic soil sequences and sequences deposited in NCBI, and were performed using maximum likelihood and Bayesian Inference methods.

Key results: We confirmed the presence of *P. tinctorius* associated with *Pinus*, *Quercus*, and *Carya illinoensis*. *Pisolithus albus*, a new record for Mexico, is distinguished by its association with *Eucalyptus* sp., a whitish exoperidium, ochraceous gleba, and echinulate basidiospores with short, isolated spines up to 1 µm high. Phylogenetic analysis supports the identity of the Mexican collections.

Conclusions: Among the Mexican samples analyzed, two well-defined species of *Pisolithus* were taxonomically identified, *P. albus* and *P. tinctorius*. Based on the material considered in this work, we did not find any that corresponded to *P. arhizus*.

Key words: barcoding, Basidiomycota, fungi from drylands, mycorrhizae.

Resumen:

Antecedentes y Objetivos: El género *Pisolithus* se caracteriza por sus basidiomas angiocárpicos, que presentan una gleba pulverulenta compuesta por peridiolos, maduración apical y basidiosporas equinuladas. En México, se han citado dos especies estrechamente relacionadas con base exclusivamente en su morfología: *Pisolithus tinctorius* y *P. arhizus*, asociadas con *Pinus*, *Quercus*, *Carya* and *Eucalyptus*. Sin embargo; se ha señalado que ninguna de estas especies se asocia con el eucalipto y *P. arhizus* se distribuye solo en Eurasia, con base en secuencias ITS. Este estudio busca contribuir a la taxonomía y filogenia del género *Pisolithus* en México mediante la caracterización de especímenes de *Pisolithus* de zonas templadas y áridas, con base en la morfología y las secuencias de código de barras ITS de especímenes de fungarios, así como secuencias de metabarcoding del suelo.

Métodos: Se caracterizaron macro y microscópicamente 32 especímenes del herbario MEXU mediante microscopía óptica y electrónica de barrido. Se utilizó literatura especializada para la identificación morfológica. El ADN se extrajo con un protocolo CTAB al 3%. La región ITS se amplificó con los pares de cebadores ITS5 e ITS4. Los análisis filogenéticos incluyeron 21 secuencias recién generadas, secuencias metagenómicas del suelo y secuencias depositadas en el NCBI, y se realizaron mediante métodos de máxima verosimilitud e inferencia bayesiana.

Resultados clave: Se confirma la presencia de *P. tinctorius* asociada con *Pinus*, *Quercus* y *Carya illinoensis*. *Pisolithus albus*, un nuevo registro para México, se distingue por su asociación con *Eucalyptus* sp., un exoperidio blanquecino, gleba ocrácea y basidiosporas equinadas con espinas cortas y aisladas de hasta 1 µm de altura. El análisis filogenético respalda la identidad de las colecciones mexicanas.

Conclusiones: Entre las muestras mexicanas analizadas, se identificaron taxonómicamente dos especies bien definidas de *Pisolithus*, *P. albus* y *P. tinctorius*. Con base en el material considerado en este trabajo, no se encontró ninguna que correspondiera a *P. arhizus*.

Palabras clave: Basidiomycota, código de barras, hongos de zonas secas, micorrizas.

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Introduction

The species included in the genus *Pisolithus* Alb. & Schwein. (Boletales: Sclerodermataceae) present globose, reniform, or pyriform basidiomes characterized by a peridium with irregular dehiscence and an alveolar gleba conformed by peridioles, which become pulverulent at maturity, with globose, echinulate basidiospores (Burgess et al., 1995). The genus is widely distributed in temperate and tropical regions, forming ectomycorrhizal associations with a wide range of woody plants, highlighting species from the families Pinaceae, Myrtaceae, Fagaceae, Dipterocarpaceae, and Cistaceae (Marx, 1977).

In Mexico, two species of *Pisolithus* have been cited: *P. arhizus* (Scop.) Rauschert. and *P. tinctorius* (Mont.) E. Fisch. The first was reported from the states of Sonora (Esqueda et al., 1990, 1998, 2000; Moreno et al., 2010; Hernández-Navarro et al., 2015), Chihuahua (Moreno et al., 2010), Hidalgo (Bautista-Hernández et al., 2018) and Jalisco (Rodríguez Alcántar et al., 2019), while the second was reported from the states of Aguascalientes (Pardavé, 1991), Chihuahua, Querétaro, Guerrero, Coahuila (Guzmán and Herrera, 1969; 1973), Mexico City (Herrera, 1964), Oaxaca (Welden and Guzmán, 1978), Veracruz (Welden and Guzmán, 1978), Hidalgo (Frutis and Guzmán, 1983), Baja California (Ayala and Guzmán, 1984), Michoacán (Díaz-Barriga et al., 1988), Chiapas (Herrera et al., 1989), Durango (Herrera et al., 1989), Morelos (Herrera et al., 1989), Sonora (Pérez-Silva et al., 1994), Mexico State (Nava Mora and Valenzuela Garza, 1997; Zarco, 1986), Nayarit, Tabasco (García-Rodríguez et al., 2006), Nuevo León (Urista et al., 1985; Garza Ocañas et al., 2023), Tamaulipas (Garza Ocañas et al., 2023), and Zacatecas (Herrera, 1964). However, in all records, the determination of the species was based exclusively on the morphological characteristics of the basidiome, considering the validity status of the species cited at the time. For this reason, both names have been indiscriminately cited worldwide (Chambers and Cairney, 1999).

The description of new species of the genus and its delimitation has been carried out using ITS barcode sequences (Martin et al., 2002, 2013; Reddy et al., 2005; Phosri et al., 2012; Rusevska et al., 2015; Lebel et al., 2018;

Abel-Aziz and Bakhit, 2023; Crous et al., 2024). Moreover, based on ITS sequences, it has been reported that *P. arhizus* is primarily distributed in Eurasia and has been introduced to South Africa (Phosri et al., 2012; Rusevka et al., 2015; Lebel et al., 2018), while *P. tinctorius* s.s. is distributed in Europe, Canada, Kenya, Nicaragua, and the USA (Díez et al., 2001; Martin et al., 2002; Lebel et al., 2018). Most records of *Pisolithus* from Mexico correspond to *Pinus-Quercus* associations (Calonge et al., 2004), with a few records from Pecan Orchards (*Carya illinoensis* (Wangenh.) K. Koch) (Sáenz-Hidalgo et al., 2023) and *Eucalyptus* L'Her. (García Rodríguez et al., 2006). However, neither *P. arhizus* nor *P. tinctorius* are associated with *Eucalyptus*, leading to the need to confirm the Mexican specimens already cited under the name "*P. arhizus*".

Our objective was to morphologically and molecularly characterize *Pisolithus* species based on collections from temperate and arid zones of Mexico preserved in the fungal collections of the herbarium MEXU. In addition, soil metabarcoding sequences were mined to integrate additional information that will contribute to the taxonomy and phylogeny of *Pisolithus* in Mexico.

Materials and Methods

Fungal material and morphological characterization

Thirty-two collections labeled as *Pisolithus* and preserved in MEXU (Appendix 1) were examined. The habitat, potential host, and collection date mentioned in the text are based on field notes or data annotated on the herbarium labels, and a distribution map (Fig. 1) was created using the ggplot2 package in R (Wickham and Chang, 2008). The specimens were photographed, measured, and characterized following the suggestions by Cifuentes et al. (1986).

Portions of the gleba were mounted in 10% KOH and observed under an OLYMPUS IX81 optical microscope (Tokyo, Japan). A portion of the gleba was metalized and visualized under Scanning Electron Microscopy (SEM) in a HITACHI SU 1510 microscope (Hitachi, Japan) in the Laboratorio de Microscopía y Fotografía de la Biodiversidad, of the Laboratorio Nacional de la Biodiversidad (LaNaBio), Instituto de Biología, Universidad Nacional Autónoma de México (IBUNAM).



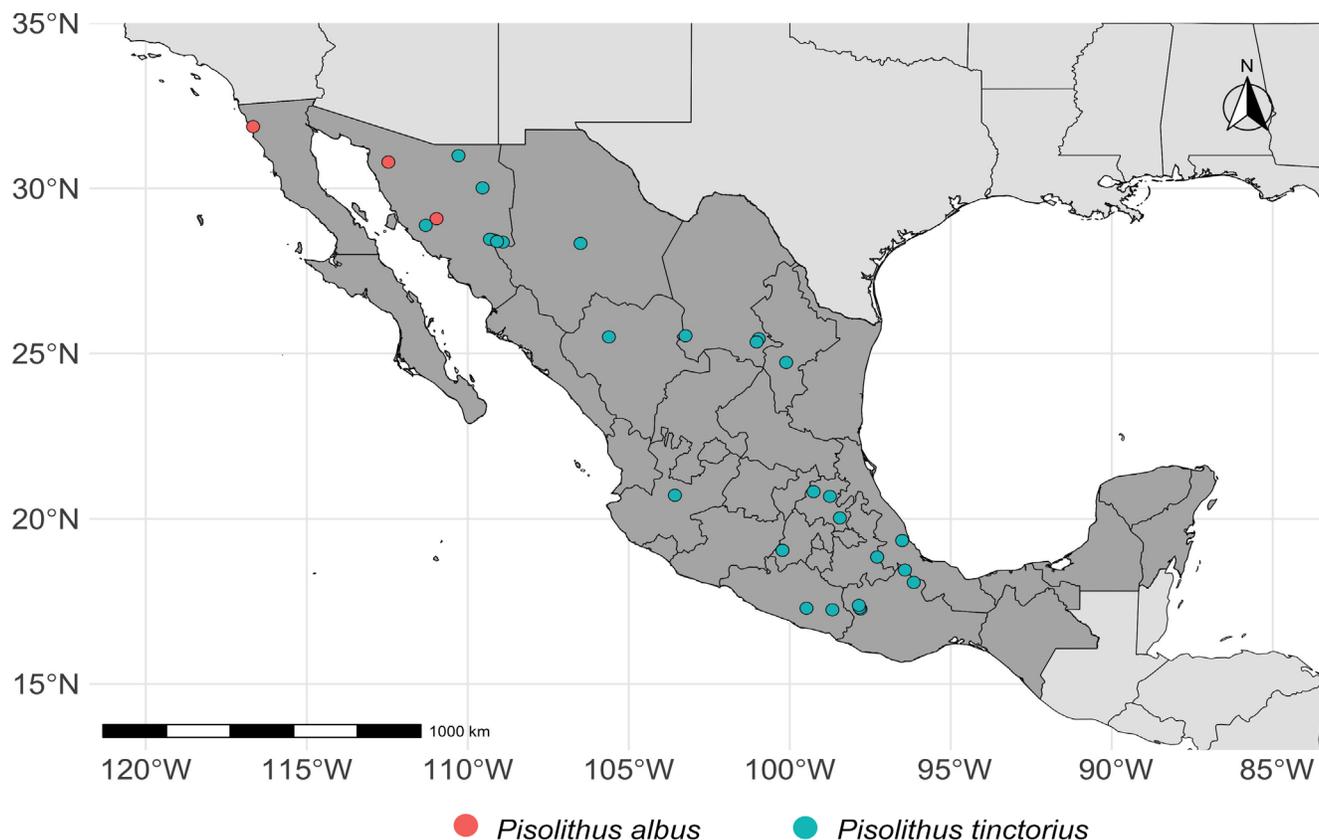


Figure 1: Distribution map of the studied material of *Pisolithus* Alb. & Scwein from Mexico.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was isolated from a small portion of the basidiome of 32 species (Appendix 1), placed in a 2 ml tube with a sterilized Tungsten sphere, frozen in liquid nitrogen, and ground with a TissueLyser Lt (QIAGEN, Hilden, Germany). After adding 500 μ l of CTAB and 2 μ l of β -mercaptoethanol per sample, the tubes were incubated in an Eppendorf ThermoMixer C (Hamburg, Germany) at 45 $^{\circ}$ C for 30 minutes at 300 rpm. Next, 500 μ l of SEVAG (chloroform: isoamyl alcohol; 24:1) was added and incubated in the Thermo Fisher Scientific Clinical Rotator (Massachusetts, USA) for 30 minutes at 85 rpm.

The mixture was centrifuged at 13,000 \times g for 10 minutes in an Eppendorf centrifuge 5424R (Hamburg, Germany). The supernatant was transferred to a 1.5 ml tube, and 500 μ l of isopropanol was added. The mixture

was gently mixed and stored at -20 $^{\circ}$ C for one hour. Subsequently, the mixture was centrifuged for 10 minutes at 12,000 \times g, and the supernatant was discarded. The pellet was washed with 70% EtOH at -20 $^{\circ}$ C, vacuum-dried for five minutes, and resuspended in 50 μ l of ultrapure water. The sample was quantified using a NanoDrop 2000 instrument (ThermoFisher Scientific, Waltham, Massachusetts, USA), and its integrity was visually verified on a 1% agarose gel stained with RedGelTM.

The ITS5 and ITS4 primer pairs were used to amplify the complete ITS1–5.8S–ITS2 region (Schoch et al., 2012) using the PCR Mix (5' BIO, Mexico). The PCR amplicons were visualized on a 1% agarose gel stained with RedGelTM. The successful amplicons underwent treatment with ExoSAP-ITTM according to the manufacturer's instructions. Clean PCR reactions were sequenced at both ends in the Laboratorio de Secuenciación Genómica of LaNaBio-IBUNAM.



Phylogenetic analysis

Twenty new sequences of *Pisolithus*, and one of *Scleroderma albidum* Pat. & Trab. were generated (Appendix 1). The ITS rDNA dataset consisted of 127 sequences and 824 positions, of which 150 were conserved, and 614 variable, 211 were singletons, and 401 were informative. For the metabarcoding soil sequences, the search was conducted in the Global Soil Mycobiome Consortium database (Tedersoo et al., 2021) using the awk command to retrieve all rows annotated as *Pisolithus* in the genus column (column 10). Only two OTU sequences were recovered: 2904dd2b5e2b1bd4c81f5402c904a0e93246127e (SOIL2904 from Nuevo León (24°43'48.0"N 100°06'36.0"W)) and 44b8a06bdba1c02587989f9e3302edb4a542d7ae0 (SOIL44b8 from Coahuila (25°21'00.0"N, 101°01'48.0"W)).

The remaining sequences were downloaded from the NCBI database (NCBI, 2024) (Appendix 2); 99 belonged to different species of *Pisolithus*, four to *Scleroderma* Pers., and one to *Astraeus hygrometricus* (Pers.) Morgan as outgroups (Anderson et al., 1998, 2001; Gomes et al., 2000; Díez et al., 2001; Malajczuk y Dunstan, 2002; Martin et al., 2002, 2013; Kanchanaprayudh et al., 2003; Thomas et al., 2003; Reddy et al., 2005; Palmer et al., 2008; Phosri et al., 2009, 2012, 2013; Jourand et al., 2010; Kasuya et al., 2008; Hitchcock et al., 2011; Wilson et al., 2011; Montagner et al., 2015; Crous et al., 2016, 2024; Lebel et al., 2018; Abdel-Aziz and Bakhit, 2023; Patel y Rajput, 2023).

The newly generated sequences were manually curated by inspecting their chromatograms in Geneious Prime v. 2025.0.3 (Geneious Prime, 2025). Sequences were aligned using the online version of MAFFT v. 7 (Kato et al., 2019). The alignments were reviewed in MESQUITE v. 3.81 (Maddison and Maddison, 2023), followed by minor manual adjustments to ensure character homology among the taxa. Phylogenetic inferences were estimated using the Maximum Likelihood Method in W-IQ-TREE (Trifinopoulos et al., 2016), and ModelFinder (Kalyaanamoorthy et al., 2017) was used to select the best substitution model. Bayesian inference analysis was conducted using MrBayes v. 3.2.6 × 64 (Huelsenbeck and Ronquist, 2001).

The information block for the matrix included two simultaneous runs, four Monte Carlo chains, a temperature

set at 0.2, and a sampling of 10 million generations (standard deviation ≤ 0.1) with trees sampled every 1000 generations. The two simultaneous Bayesian runs continued until convergence parameters were met, and the standard deviation fell below 0.0001 after 10 million generations. The final tree was edited using FigTree v. 1.4.4 (Rambaut, 2018).

Results

The 32 collections studied and preserved under *Pisolithus* come from 23 municipalities of 12 states of the Mexican Republic (Fig. 1, Appendix 1). After examining the collections, we determined that the specimens correspond to two well-defined species: *Pisolithus albus* (Cooke & Massee) Priest, a new record for Mexico, associated with *Eucalyptus* in the semiarid zones of Northwest Mexico, and *P. tinctorius* associated with *Pinus* spp., *Quercus* spp., and *Carya illinoensis*, with a broader distribution in our country.

We successfully amplified and sequenced the ITS region of 21 *Pisolithus* collections, and the data of Tedersoo et al. (2021) resulted in two consensus sequences of *P. tinctorius* from Coahuila and Nuevo León. One collection (MEXU 11889) deposited under the name "*P. arhizus*" was analyzed using both molecular and morphological methods. After our examination, we concluded that this collection corresponds to *Scleroderma albidum*.

The result of Posterior Probability (PP) from Bayesian inference and bootstrap from maximum likelihood (BS) generated trees with similar topologies; the topology of the Bayesian analysis is shown (Fig. 2). The genus *Pisolithus* recovered as monophyletic (PP/BS=0.70/92). It presents three distinct lineages: the first comprises seven species (PP/BS=0.74/90): *P. arhizus*, *P. capsulifer* (Sowerby) Watling, Phosri & M.P. Martín, *P. calongei* M.P. Martín, Phosri & Watling, *P. hypogaeus* S.R. Thomas, Dell & Trappe, *P. marmoratus* (Berk.) E. Fisch, *P. orientalis* Watling, Phosri & M.P. Martín and *P. tinctorius*. Most sequences generated in this work grouped with *P. tinctorius* with high support (PP/BS=0.99/100) and are associated with *Pinus* spp., *Quercus* spp., and *Carya illinoensis*. The second lineage includes five species (PP/BS=0.97/95): *P. albus*, *P. croceorrhizus* P.



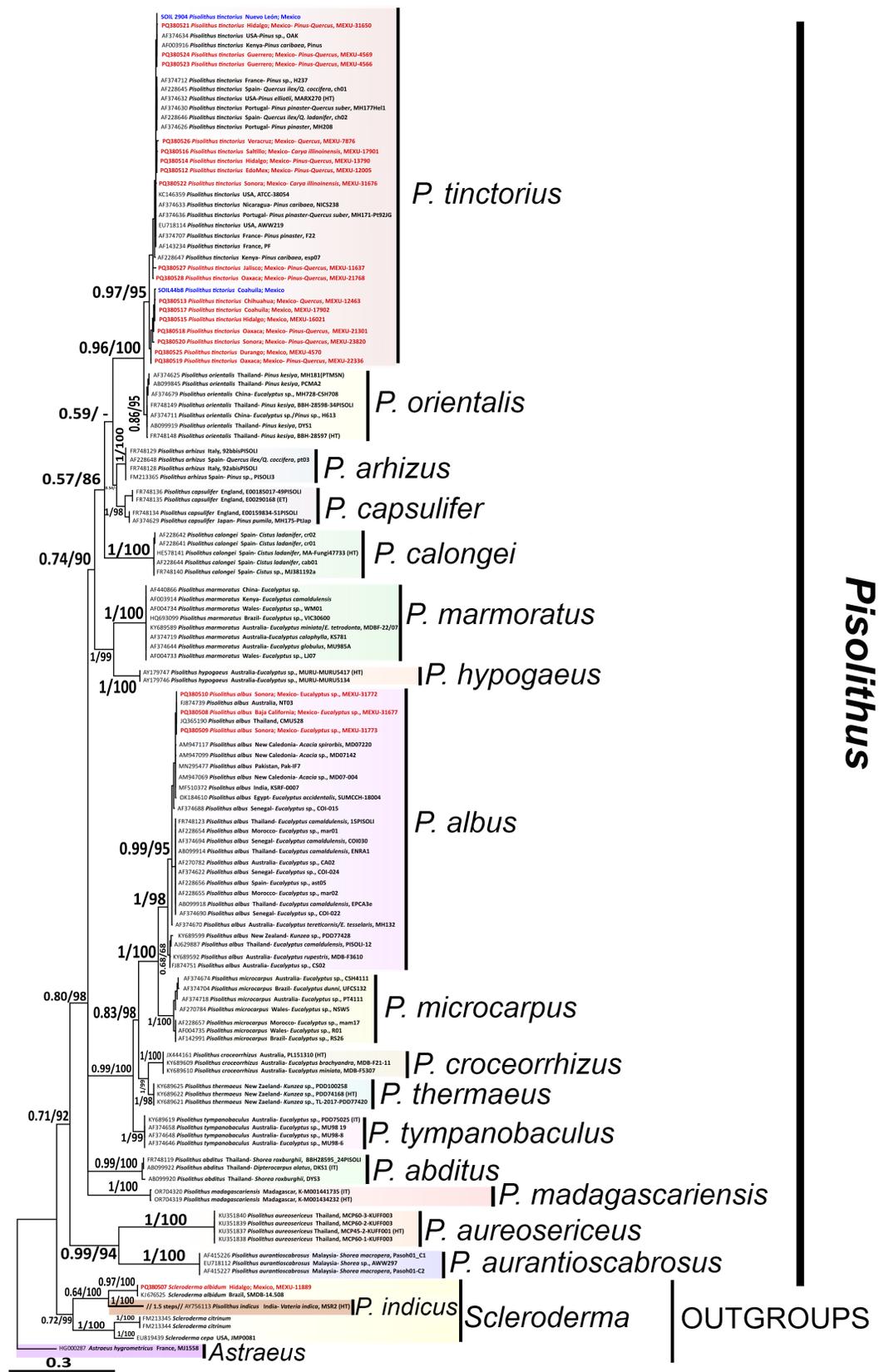


Figure 2: Phylogram of Bayesian inference (BI) tree from the ITS sequence data of *Pisolithus* Alb. & Schwein. The numbers above branches represent Bayesian Posterior Probabilities (PP=0-1), and Bootstrap Values (BS=0-100) for Maximum Likelihood. The scale bar represents the expected number of nucleotide substitutions per site. The taxa whose sequences have been obtained in this study are marked in bold red. Metagenomic soil sequences are marked in blue. Accession numbers of NCBI (2024) are indicated in each sequence.



Leonard & McMull.-Fish., *P. microcarpus* (Cooke & Masee) G. Cunn., *P. thermaeus* T. Lebel, Pennycook & Beever, and *P. tympanobaculus* T. Lebel & M.D. Barrett. Three sequences generated in this study grouped with *P. albus* (PP/BS=0.99/95), a new record for Mexico. Two species form an unresolved polytomy: *P. abditus* Kanch., Sihan., Hogetsu & Watling, and *P. madagascariensis* Rivas-Ferreiro, Dentinger, Suz & A.M. Ainsw. The third lineage (PP/BS=0.99/94) is composed of *P. aureosericeus* M.P. Martín, Kaewgraj., Phosri & Watling, and *P. aurantioscabrosus* Watling. The *P. indicus* sequence was grouped within the *Scleroderma* genus (PP/BS=0.6/100).

Taxonomy

Basidiomycota

Agaricomycetes

Boletales

Sclerodermataceae

Pisolithus albus (Cooke & Masee) Priest, in Lebel, Pennycook and Barrett, *Phytotaxa* 348(3): 167. 2018. Fig. 3.

≡ *Polysaccum album* Cooke and Masee, *Grevillea* 20 (no.94): 36. 1891.

TYPE: AUSTRALIA. Queensland, Dundoo, on the ground, undated, *Martin 916* (holotype K!).

Basidiome epigeous or semi-epigeous, 45-110 mm wide × 65-190 mm tall × 30-120 mm diameter, subglobose, ovoid to pyriform, with a robust and rooted base; peridium thin, smooth, cracking into irregular segments from top to bottom at maturity, whitish when young, cream or beige when mature; gleba conformed into peridioles 2-5 mm diameter, subglobose to ovoid, cream-colored when young and turning ochre to olivaceous when mature; basidiospores 7-11 μm, globose, echinulate, bright yellowish, in SEM isolated straight to slightly curved spines up to 1 μm high; basidia not observed.

Habit: on soil, associated with *Eucalyptus* spp.

Distribution: Africa: Egypt (Abdel-Aziz and Bakhit, 2023), Morocco (Eddine Bakkali Yakhlef et al., 2009), Senegal (Duponnois and Bâ, 1999), Tunisia (Jaouani et al., 2015). America: Mexico (this study), USA (Phosri et al., 2012). Eurasia: India (Singla et al., 2004), Italy (Gargano et al., 2018), Malaysia (Martin et al., 2002), Spain (Díez et al., 2001), Thailand (Kanchanaprayudh et al., 2003). Oceania: Australia (Lebel et al., 2018), New Caledonia (Hosaka, 2009), New Zealand (Moyersoen et al., 2003).

Studied material: MEXICO. Baja California, municipio Ensenada, 31°52'12.9"N, 116°39'59.0"W, under *Eucalyptus* sp., 25.I.2019, *E. Hernández-Navarro 774* (MEXU 31677). Sonora, municipio Caborca, 30°47'46"N, 112°27'56"W, under *Eucalyptus* sp., 18.IV.2019, *R. Gutierrez and A. Gutierrez s.n.* (MEXU 31773). Municipio Hermosillo, 29°04'53"N, 110°58'07"W, under *Eucalyptus* sp., 06.IX.1995, *M. Esqueda s.n.* (MEXU 31772).

Notes: *Pisolithus albus* is recognized by its white exoperidium, olivaceous gleba, and basidiospores 7-11 μm diameter with isolated, low, straight spines. The *P. albus* specimens in this study were always collected under *Eucalyptus* sp.; however, this species is also associated with *Acacia s.l.* and *Kunzea* Benth. (Abdel-Aziz and Bakhit, 2023). This is the first record of *P. albus* from Mexico. Mexican specimens, mainly from Caborca, Sonora, are stouter (up to 19 × 11 cm diameter) than reported in the holotype (5-6 cm diameter) (Cooke, 1891). However, materials from Italy are 3-20 cm diameter (Gargano et al., 2018), while those from Tunisia are 3-12 cm diameter (Jaouani et al., 2015), and those from Egypt are 4.5-15 × 6-18 cm diameter (Abdel-Aziz and Bakhit, 2023). Additionally, in the holotype, basidiospores are 9-10 μm diameter, while our materials are 7-11 μm diameter; however, it matches with Egyptian materials (7-11 μm diameter), while Tunisians are 9-12 μm diameter; Italians 8.75-10.25 μm diameter (Jaouani et al., 2015; Gargano et al., 2018; Abdel-Aziz and Bakhit, 2023).

Young specimens of *P. albus* may recall *P. croceorrhizus*, as both species are commonly associated with *Eucalyptus* trees. However, *P. croceorrhizus* has a



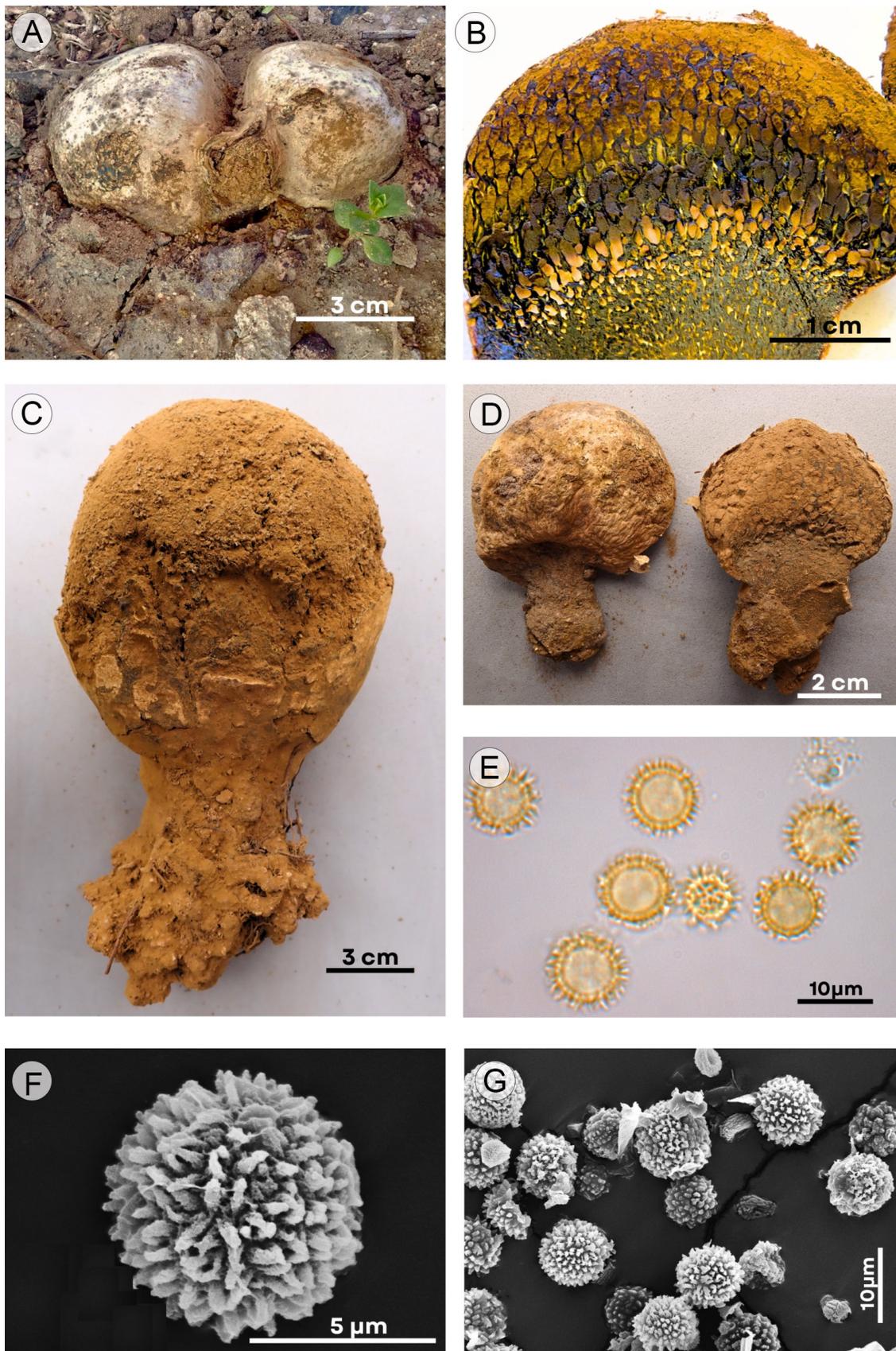


Figure 3: *Pisolithus albus* (Cooke & Masee) Priest. A. specimen in its habitat (MEXU 31677); B. peridioles (MEXU 31677); C-D. herbarium specimens (MEXU 31772); E. basidiospores in light microscopy (MEXU 31772); F.-G. basidiospores in SEM (MEXU 31677).

distribution practically restricted to Australia (Southeast Queensland) and New Caledonia. Moreover, *P. albus* presents larger basidiospores (7-12 µm diameter) with irregular pyramidal spines. In contrast, *P. croceorrhizus* presents smaller basidiospores (5.3-6.9 µm diameter) densely covered with pyramidal spines connected basally in a low reticulum (Leonard et al., 2013).

Pisolithus tinctorius (Mont.) E. Fisch., in Engler and Prantl, Nat. Pflanzenfam., Teil I (Leipzig) 1: 338 (1900). Fig. 4.

=*Polysaccum tinctorium* Mont., in Webb and Berthelot, Hist. nat. Iles Canar. (Paris) 3(2): 87. 1840.

=*Lycoperdodes canariense* Kuntze, Revis. gen. pl. (Leipzig) 2: 859. 1891.

TYPE: SPAIN. Canary Islands, without precise location, at the roots of *Cistus*, s.f., coll. Despréaux s.n. (holotype: not located).

Basidiomes epigeous, globose, claviform to irregularly shaped, subglobose, ovoid or pyriform, 35-90 mm wide × 55-150 mm high × 30-120 mm diameter, with a robust or sometimes sessile base; peridium thin, papery, smooth, and brittle, yellowish-whitish when young, brown when mature; dehiscence through irregular cracks; gleba reddish-brown to dark brown, structured in peridioles that become powdery with maturity; peridioles 2-5 mm, subglobose, ovoid or irregular and somewhat angular, creamy when young and becoming yellowish when mature, smaller and more compact towards the stipe; basidiospores 8.5-12 µm, globose, strongly echinulate, dark ochre; in SEM spines primarily straight, coalescent, some slightly curved towards the apex; basidia not observed.

Habit: on soil, associated with *Pinus-Quercus* and *Carya illinoensis*.

Distribution: Africa: Kenya (Martin et al., 1998), South Africa (Martin et al., 2002). America: USA (Gomes et al., 2000; Martin et al., 2002; Phosri et al., 2012), Mexico (Herrera, 1964; this study), Nicaragua (Martin et al., 2002).

Eurasia: France (Gomes et al., 2000), Philippines (Anderson et al., 2001), Portugal (Martin et al., 2002), Spain (Díez et al., 2001; Phosri et al., 2012).

Studied material: MEXICO. Chihuahua, municipio Cd. Cuauhtémoc, km 67 Chihuahua - Ciudad Cuauhtémoc road, 28°26'57.8"N, 106°52'10.1"W, under *Quercus*, 25.IX.1978, E. Pérez-Silva y R. Hernández s.n. (MEXU 12463). Ciudad de México, alcaldía Cuajimalpa de Morelos, Desierto de los Leones, 2900 m, 19°17'51.4"N, 99°18'56.0"W, under *Pinus* sp., 02.VII.1950, T. Herrera s.n. (MEXU 1409). Coahuila, municipio Saltillo, 25°26'56.3"N, 100°57'44.4"W, under *Carya illinoensis*, 10.VIII.1982, T. Herrera s.n. (MEXU 17901). Municipio Torreón km 22 Torreón - Matamoros road, 25°32'18.9"N, 103°14'13.3"W, agricultural land, 12.VIII.1982, T. Herrera s.n. (MEXU 17902). Durango, municipio Tepehuanes, Sierra de la Candela, 25°30'10.6"N, 105°36'56.9"W, 17.IX.1960, F. Sánchez s.n. (MEXU 4570). Estado de México, municipio Temascaltepec, 1100 m, 19°02'37.1"N, 100°13'30.2"W, in *Pinus-Quercus* forest, 12.I.1978, R. Hernández s.n. (MEXU 12005). Guerrero, municipio Chilpancingo de los Bravo, Rincón Viejo, 17°14'40.5"N, 98°40'30.2"W, in *Pinus-Quercus* forest, 13.VI.1963, H. Kruse s.n. (MEXU 4569); 17°17'28.8"N, 99°28'46.0"W, in *Pinus-Quercus* forest, 21.VI.1963, H. Kruse s.n. (MEXU 4566). Hidalgo, municipio Cardonal, 45 km east of Ixmiquilpan, 2000 m, 20°38'53.4"N, 98°57'52.5"W, 02.X.1980, R. Hernández y D. Rodríguez s.n. (MEXU 16021). Municipio Metztlán, 20°40'30"N, 98°45'09"W, in *Pinus-Quercus* forest, 11.X.2023, E. Hernandez-Navarro 720 (MEXU 31650). Municipio Santiago Tulantepec de Lugo Guerrero, 2370 m, 20°03'35.5"N, 98°26'07.3"W, in *Pinus-Quercus* forest, 03.VII.1979, R. Hernández s.n. (MEXU 13200). Municipio Zimapán, La Majada, 20°49'08.6"N, 99°15'33.6"W, in *Pinus-Quercus* forest, 06.X.1979, R. Hernández s.n. (MEXU 13790). Jalisco, municipio Zapopan, Cañón de las Flores, km 25 Guadalajara - Tequila road, 1500 m, 20°40'27.9"N, 103°23'37.1"W, in *Pinus-Quercus* forest, 25.VIII.1977, R. Hernandez s.n. (MEXU 11637). Oaxaca, municipio Acatlán de Pérez Figueroa, 2100 m, 18°26'44.8"N, 96°25'18.0"W, in *Pinus-Quercus* forest, 23.IV.1976, E. Pérez-Silva et al. s.n. (MEXU 10485); La Carbonera, 19°02'37.1"N, 100°13'30.2"W, in *Pinus-Quercus* forest, 23.VI.1976, E. Pérez-Silva et al. s.n. (MEXU 10996).



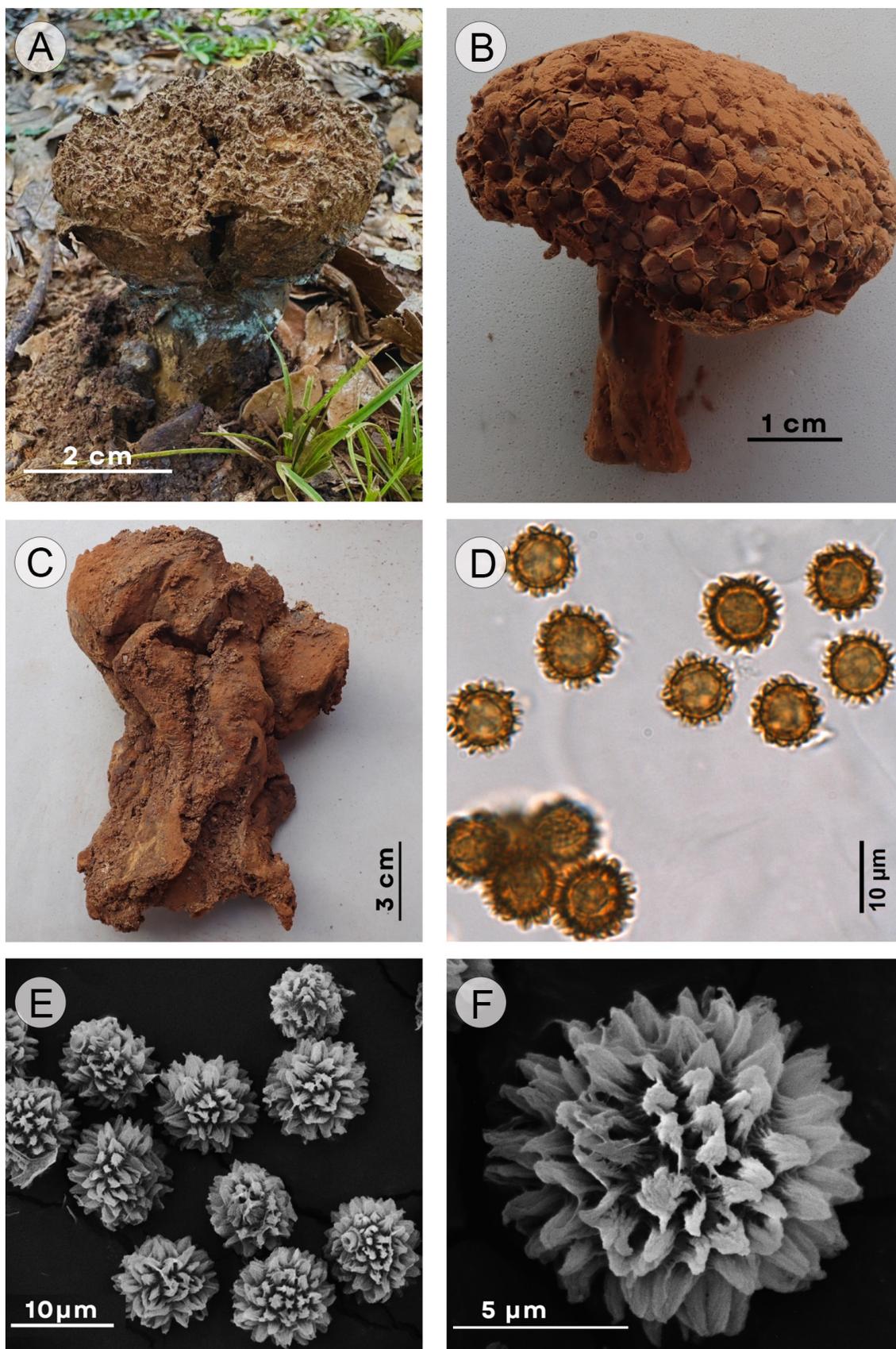


Figure 4: *Pisolithus tinctorius* (Mont.) E. Fisch. A. specimen in its habitat (MEXU 31650); B-C. herbarium specimens (MEXU 4569, MEXU 13790); D. basidiospores in light microscopy (MEXU17902); E-F. basidiospores in SEM (MEXU-31650).

Municipio San Juan Mixtepec, 8 km from la Cañada El Águila, 1900-2250 m, 17°16'09.6"N, 97°48'07.2"W, in *Pinus-Quercus* forest, 03.X.1989, E. Pérez-Silva et al. s.n. (MEXU 22336); 17°18'20.5"N, 97°49'54.7"W, under *Pinus-Quercus*, 02.X.1988, J. Reyes-Santiago s.n. (MEXU 21301); Cerro del Rio Azucena, 2000 m, 17°22'52.1"N, 97°51'20.9"W, *Pinus-Quercus* forest, 21.VII.1989, J. Reyes Santiago s.n. (MEXU 21768). Municipio Tuxtepec, 18°04'20.3"N, 96°08'52.8"W, under *Pinus caribaea*, 31.I.1974, R. Singer s.n. (MEXU 8969). Sonora, municipio Cananea, 30°59'23.6"N, 110°17'19.9"W, in *Pinus-Quercus* forest, 10.IX.1993, E. Pérez-Silva y M. Esqueda s.n. (MEXU 23820); km 18 Santa Rosa - Yécora road, 28°26'03.8"N, 109°10'34.3"W, under *Quercus* sp., 05.VIII.1989, A. Aparicio s.n. (MEXU 22604). Municipio Hermosillo, 28°52'48.8"N, 111°18'14.2"W, under *Carya illinoensis*, 03.X.2019, E. Hernández-Navarro 746 (MEXU 31676). Municipio Yécora, Rincón de Güerigo, 28°26'03.8"N, 109°10'34.3"W, under *Quercus* sp., 26.VII.1989, A. Aparicio s.n. (MEXU 22605); 28°23'41"N, 109°05'26"W, under *Pinus* sp., 10.IX.1995, M. Esqueda et al. s.n. (MEXU 31778); 28°23'41"N, 109°05'26"W, *Pinus-Quercus* forest, 13.IX.1996, M. Esqueda et al. s.n. (MEXU 31777). Veracruz, municipio Maltrata, Cumbres de Maltrata, a 1700 m, 18°50'21.2"N, 97°17'08.8"W, under *Quercus*, 28.VIII.1969, R. Hernandez y R. C. Trigos s.n. (MEXU 7877); Cumbres de Maltrata, a 1600 m, 18°50'21.2"N, 97°17'08.8"W, under *Quercus*, 28.VIII.1969, R. Hernández y R. C. Trigos s.n. (MEXU 7876). Municipio Emiliano Zapata, 950 m, 19°20'29.3"N, 96°30'33.0"W, under *Quercus*, 10.VII.1974, R. Hernández s.n. (MEXU 9575).

Notes: *Pisolithus tinctorius* could be confused with *P. arhizus*, which has the same spore size and is associated with *Pinus* L. and *Quercus* L. plants. Rusevska et al. (2015) mention that *P. arhizus* and *P. tinctorius* are so similar that it is almost impossible to separate these species morphologically. However, based on materials from Macedonia, Rusevska et al. (2015) mention that *P. tinctorius* presents a shorter pseudostipe (40-50 × 20-25 mm), a very thin yellowish olivaceous peridium, and basidiospores with spines on average of 1.6 µm long, more or less isolated; while *P. arhizus* presents a larger pseudostipe (≤95 mm × (20-)25-

40(-50) mm), pale ochre to brown black peridium, and the spines of the basidiospores are smaller in average (1.2 µm), isolated but very compact, in parts forming small pyramids (Rusevska et al., 2015). Nevertheless, molecular data support two distinct taxa restricted in distribution in the northern hemisphere: *P. tinctorius* s.s. is distributed in Europe and North America, while *P. arhizus* occurs in Eurasia and has been introduced in South Africa (Lebel et al., 2018).

The species *P. tinctorius* could also be confused with *P. marmoratus*, as they share the size of their basidiospores and spines. However, the latter is associated with *Eucalyptus* and is distributed in the southern hemisphere (Martin et al., 2002). Additionally, *P. marmoratus* presents a peridium with black warts and black pseudostipes (Leonard et al., 2013).

Discussion

We present the first formal taxonomic study of *Pisolithus* in Mexico based on morphological and molecular data of fungarium specimens. According to Index Fungorum (2024), the genus currently includes 19 valid species. However, some controversies persist regarding the correct identification, nomenclature, and possible synonymy of some species.

Since the description of *Pisolithus*, with *P. arenarius* Alb. & Schwein. as the type species, Albertini and Schweinitz (1805) denoted the lack of detailed information on closely related species, then placed in *Scleroderma* such as *Scleroderma arhizum* (Scop.) Pers. (= *P. arhizus*) and *S. tinctorium* Pers. (= *P. tinctorius*), to which *P. arenarius* most closely resembled. Although *P. arenarius* is a valid species, it still requires more up-to-date information on its macro-morphological variation, and specimens in various herbaria have not been reexamined or sequenced for verification.

For a long time, *P. arhizus* was considered conspecific with *P. tinctorius*. Consequently, both species were cited interchangeably worldwide (Chambers and Cairney, 1999). Later, phylogenetic studies using ITS barcode sequences confirmed them as distinct species: *P. arhizus* is primarily distributed in Eurasia and introduced to South Africa, and *P. tinctorius* s.s. is found in Europe and North America (Leonard et al., 2013; Martín et al., 2013).



Numerous fungal collections and sequences in public databases require updating (Lebel et al., 2018). *Pisolithus tinctorius* originally was described under the genus *Polysaccum* Mont., associated with *Cistus* L. from the Canary Islands; however, global identification of the species has primarily followed Coker and Couch (1928), focusing on samples related to *Pinus-Quercus* forest, but *P. tinctorius* (Pers.: Pers.) Coker & Couch turned out to be an illegitimate name for a taxon nowadays recognized as *P. arhizus*.

In the case of *P. kisslingii* E. Fisch., no barcode sequences or recent collections exist, while *P. indicus* Natarajan & Senthil is represented by a single sequence in NCBI (2024) which, in our analyses, grouped with *Scleroderma*. This species was described from India as having grayish gleba, a characteristic not reported in any other *Pisolithus* species (Reddy et al., 2005). The combination of characters and the sequence analysis suggests that this could be a *Scleroderma* species. Additionally, Reddy et al. (2005) did not provide photographs of the peridioles, no sequences of *Scleroderma* were included in the analysis, and the clade containing *P. indicus* grouped with the rest of the *Pisolithus* species with low support (BS= 36). Leonard et al. (2013) included the *P. indicus* sequence, which was grouped with *P. aurantioscabrosus*. Nonetheless, no *Scleroderma* sequences were included.

Lebel et al. (2018) included some LSU sequences of *P. albus*, *P. croceorrhizus*, and *P. indicus*; the latter was placed as a basal taxon, paraphyletic to *P. aurantioscabrosus* and *P. aureosericeus*. Nevertheless, they did not include any *Scleroderma* sequences in the analysis. On the other hand, most published phylogenies present low support values in the deepest branches of the genus, as only ITS sequences have been analyzed, except for the one proposed by Lebel et al. (2018), which included some LSU sequences. Hence, other markers, such as EF1 α or RPB1, are needed to resolve these ambiguities within the genus.

In our studied materials, *P. tinctorius* was primarily collected from *Pinus-Quercus*, except for two collections found under *Carya illinoensis* (MEXU 17901; 31676). Both collections originate from arid zones transformed into commercial pecan agricultural lands with abundant

canopy, organic matter, fertilization, and irrigation, which favor the development of *P. tinctorius* despite the high temperatures of the zone. This aligns with reports that *P. tinctorius* can be associated with *C. illinoensis* (Sáenz-Hidalgo et al., 2023). This is due to soil and plant inoculation practices, as *P. tinctorius* has historically been used in forestry to promote tree growth (García-Rodríguez et al. 2006). As a result, it has been introduced globally (Lebel et al., 2018). This adaptability explains its presence in diverse environments, including acidic soils and areas with heavy metal contamination (Lampky and Peterson, 1963; Sebastianiana et al., 2020).

Regarding *P. albus*, a new record for Mexico, the specimens from Sonora and Baja California, whose sequences are similar to those from Senegal and Egypt, are also associated with *Eucalyptus* spp., a widely distributed introduced genus (Abdel-Aziz and Bakhit, 2023). Hence, the presence of other *Pisolithus* species in unexplored regions of Mexico is possible. For example, García-Rodríguez et al. (2006) reported collections of *P. tinctorius* from *Eucalyptus* plantations, but no barcode sequences were generated; ergo, their specimens are likely to correspond with another species, since *P. tinctorius* does not form associations with *Eucalyptus*. These reports support the need for further sampling across Mexico, primarily in underexplored arid and semiarid zones where gasteroid fungi are more diverse and abundant, as well as areas with vegetal species that match the known host of the genus.

Of the 32 collections reviewed, 21 were successfully sequenced; however, DNA degradation posed challenges due to specimen age, with collections ranging from 1 to 74 years old (average age: 45 years). Future collections should prioritize conditions that preserve DNA integrity for molecular analyses. This work underscores the need for continued research on *Pisolithus* to achieve comprehensive species diversity in Mexico.

Conclusions

We confirm the presence of two species of *Pisolithus* widely distributed in Mexico. *Pisolithus tinctorius* is mainly associated with *Pinus-Quercus* trees and *Carya illinoensis* trees. Until now, its presence has been molecularly



confirmed in the states of Chihuahua, Coahuila, Durango, Guerrero, Hidalgo, Jalisco, the State of Mexico, Oaxaca, Sonora, and Veracruz, while *P. albus* is associated with *Eucalyptus* in semiarid zones of northwestern Mexico, in Sonora and Baja California. Among the specimens studied from Mexico and soil metabarcoding sequences, we had no evidence of any that could be identified with *P. arhizus*.

Authors contributions

EHN conceived the main research, collected specimens, and elaborated the map. ISI performed molecular protocols and elaborated the rest of the plates. Both authors morphologically characterized the specimens, performed the bioinformatic protocols, and prepared the final manuscript.

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Literature cited

Abdel-Aziz, A. E. and M. S. Bakhit. 2023. *Pisolithus albus* (Sclerodermataceae), a first record from Egypt. *Plant and Fungal Systematics* 68(1): 232-238. DOI: <https://doi.org/10.35535/pfsyst-2023-0004>

Albertini, J. B. and L. D. Schweinitz. 1805. *Conspectus Fungorum in Lusatae Superioris Agro Niskiensi Crescentium e Methodo Persooniana. Sumtibus Kummerianis*. Leipzig, Germany. Pp. 376. DOI: <https://doi.org/10.5962/bhl.title.3601>

Anderson, I. C., S. M. Chambers and J. Cairney. 1998. Molecular determination of genetic variation in *Pisolithus* isolates from a defined region in New South Wales, Australia. *New Phytologist* 138(1): 151-162. DOI: <https://doi.org/10.1046/j.1469-8137.1998.00894.x>

Anderson, I. C., S. M. Chambers and J. W. G. Cairne. 2001. ITS-RFLP and ITS sequence diversity in *Pisolithus* from central and eastern Australian sclerophyll forests. *Mycological Research* 105(11): 1304-1312. DOI: <https://doi.org/10.1017/S0953756201005044>

Ayala, N. and G. Guzmán. 1984. Los hongos de la Península de Baja California, I. Las especies conocidas. *Boletín de Sociedad Mexicana de Micología* 19: 73-91.

Bautista-Hernández, S., T. Raymundo, E. Aguirre-Acosta, M. Contreras-Pacheco, L. Romero-Bautista and R. Valenzuela. 2018. Agaricomycetes gasteroides del bosque mesófilo de montaña de la Huasteca Alta Hidalguense, México. *Acta Botanica Mexicana* 123: 21-36. DOI: <https://doi.org/10.21829/abm123.2018.1266>

Burgess, T., N. Malajczuk and B. Dell. 1995. Variation in *Pisolithus* based on basidiome and basidiospore morphology, culture characteristics and analysis of polypeptides using 1D-SDS-PAGE. *Mycological Research* 99(1): 1-13. DOI: [https://doi.org/10.1016/S0953-7562\(09\)80309-2](https://doi.org/10.1016/S0953-7562(09)80309-2)

Calonge, F. D., G. Guzmán and F. Ramírez-Guillén. 2004. Observaciones sobre los Gasteromycetes de México depositados en los Herbarios XAL y XALU. *Boletín de la Sociedad Micológica de Madrid* 28: 337-371.

Chambers, S. M. and J. W. Cairney. 1999. *Pisolithus*. In: Cairney, J. W. and S. M. Chambers (eds.). *Ectomycorrhizal fungi. Key genera in profile*. Springer. Berlin, Germany. Pp. 1-31.

Cifuentes, J., M. Villegas and L. Pérez-Ramírez. 1986. Hongos. In: Lot, A. and F. Chiang (eds.). *Manual de Herbario. Consejo Nacional de la Flora de México, A.C. México, D.F., México*. Pp. 55-64.

Coker, W. C., and J. N. Couch. 1928. *The Gasteromycetes of the Eastern United States and Canada*. Chapel Hill, The University of North Carolina Press. Chapel Hill, USA. 195 pp.

Cooke, M. C. 1891. *Australian Fungi*. *Grevillea* 20(93): 4-7.

Crous, P. W., M. J. Wingfield, D. M. Richardson, J. J. Leroux, D. Strasberg, J. Edwards, F. Roets, V. Hubka, P. W. J. Taylor,



- M. Heykoop, M. P. Martín, G. Moreno, D. A. Sutton, N. P. Wiederhold, C. W. Barnes, J. R. Carlavilla, J. Gené, A. Giraldo, V. Guarnaccia, J. Guarro, M. Hernández-Restrepo, M. Kolařík, J. L. Manjón, I. G. Pascoe, E. S. Popov, M. Sandoval-Denis, J. H. C. Woudenberg, K. Acharya, A. V. Alexandrova, P. Alvarado, R. N. Barbosa, I. G. Baseia, R. A. Blanchette, T. Boekhout, T. I. Burgess, J. F. Cano-Lira, A. Čmoková, R. A. Dimitrov, M. Yu. Dyakov, M. Dueñas, A. K. Dutta, F. Esteve-Raventós, A. G. Fedosova, J. Fournier, P. Gamboa, D. E. Gouliamova, T. Grebenc, M. Groenewald, B. Hanse, G. E. ST. J. Hardy, B. W. Held, Ž. Jurjević, T. Kaewgrajang, K. P. D. Latha, L. Lombard, J. J. Luangsa-ard, P. Lysková, N. Mallátová, P. Manimohan, A. N. Miller, M. Mirabolfathy, O. V. Morozova, M. Obodai, N. T. Oliveira, M. E. Ordóñez, E. C. Otto, S. Paloi, S. W. Peterson, C. Phosri, J. Roux, W. A. Salazar, A. Sánchez, G. A. Sarria; H. D. Shin, B. D. B. Silva, G. A. Silva, M. TH. Smith, C. M, Souza-Motta, A. M. Stchigel, M. M. Stoilova-Disheva, M. A. Sulzbacher, M. T. Telleria, C. Toapanta, J. M, Traba, N. Valenzuela-Lopez, R. Watling and J. Z. Groenewald. 2016. Fungal Planet description sheets: 400-468. *Persoonia-Molecular Phylogeny and Evolution of Fungi* 36(1): 316-458. DOI: <https://doi.org/10.3767/003158516X692185>
- Crous, P. W., Ž. Jurjević, S. Balashov, S. De la Peña-Lastra, A. Mateos, U. Pinruan, A. Rigueiro-Rodríguez, E. R. Osieck, A. Altés, P. Czachura, F. Esteve-Raventós, S. Gunaseelan, M. Kaliyaperumal, E. Larsson, J. J. Luangsa-ard, G. Moreno, F. Pancorbo, M. Piątek, S. Sommai, S. Somrithipol, M. Asif, G. Delgado, A. Flakus, T. Illescas, K. Kezo, P. Khamsuntorn, A. Kubátová, R. Labuda, C. Lavoise, T. Lebel, P. Lueangjaroenkit, J. G. Maciá-Vicente, A. Paz, M. Saba, R. G. Shivas, Y. P. Tan, M. J. Wingfield, T. Aas, B. Abramczyk, A. M. Ainsworth, A. Akulov, P. Alvarado, F. Armada, B. Assyov, R. Avchar, M. Avesani, J. L. Bezerra, J. D. Bhat, P. Bilański, D. S. Bily, F. Boccardo, F. Bozok, J. C. Campos, S. Chaimongkol, N. Chellappan, M. M. Costa, M. Dalecká, V. Darmostuk, V. Daskalopoulos, J. Dearnaley, B.T.M. Dentinger, N.I. De Silva, D. Dhotre, J. R. Carlavilla, C. Doungsa-ard, F. Dovana, A. Erhard, L. O. Ferro, S. C. Gallegos, C. E. Giles, G. Gore, M. Gorfer, F. E. Guard, S.-Å. Hanson, P. Haridev, R. Jankowiak, S. N. Jeffers, H. Kandemir, A. Karich, K. Kisto, L. Kiss, I. Krisai-Greilhuber, K. P. D. Latha, M. Lorenzini, S. Lumyong, P. Manimohan, J. L. Manjón, F. Maula, E. Mazur, N. L. S. Mesquita, K. Młynek, S. Mongkolsamrit, P. Morán, R. Murugadoss, M. Nagarajan, S. Nalumpang, W. Noisripoom, S. Nosalj, Q. S. Novaes, M. Nowak, J. Pawłowska, M. Peiger, O. L. Pereira, A. Pinto, M. Plaza, E. Polemis, A. Polhorský, D. O. Ramos, M. Raza, M. Rivas-Ferreiro, P. Rodriguez-Flakus, M. Ruszkiewicz-Michalska, A. Sánchez, A. Santos, A. Schüller, P. A. Scott, İ. Şen, D. Shelke, L. Śliwa, H. Solheim, H. Sonawane, D. Strašitáková, M. Stryjak-Bogacka, M. Sudsanguan, N. Suwannarach, L. M. Suz, K. Syme, H. Taşkın, D. S. Tennakoon, P. Tomka, N. Vaghefi, V. Vasan, J. Vauras, D. Wiktorowicz, M. Villarreal, A. Vizzini, M. Wrzosek, X. Yang, W. Yingkunchao, G. Zapparoli, G. I. Zervakis and J. Z. Groenewald. 2024. Fungal Planet description sheets: 1614-1696. *Fungal systematics and evolution* 13(1): 183-440. DOI: <https://doi.org/10.3114/fuse.2024.13.11>
- Díaz-Barriga, H., F. Guevara-Féfer and R. Valenzuela. 1988. Contribución al conocimiento de los macromicetos del estado de Michoacán. *Acta Botanica Mexicana* 2: 21-44. DOI: <https://doi.org/10.21829/abm2.1988.564>
- Díez, J., B. Anta, J. L. Manjón and M. Honrubia. 2001. Genetic variation of *Pisolithus* isolates associated with native hosts and exotic eucalyptus in the western Mediterranean region. *New Phytologist* 149(3): 577-587. DOI: <https://doi.org/10.1046/j.1469-8137.2001.00036.x>
- Duponnois, R. and A. M. Bâ. 1999. Growth stimulation of *Acacia mangium* Willd by *Pisolithus* sp. in some Senegalese soils. *Forest Ecology and Management* 119(1-3): 209-215. DOI: [https://doi.org/10.1016/S0378-1127\(98\)00524-6](https://doi.org/10.1016/S0378-1127(98)00524-6)
- Eddine Bakkali Yakhlef, S., Mousain, R. Duponnois, M. Ducousso, A. Belkouri, B. Kerdouh, M. M. Perrineau and M. Abouroh. 2009. Molecular phylogeny of *Pisolithus* species from Moroccan forest woodlands. *Symbiosis* 49: 157-162. DOI: <https://doi.org/10.1007/s13199-009-0043-9>
- Esqueda, M., T. Quintero Ruiz, E. Pérez-Silva and A. Aparicio Navarro. 1990. Nuevos registros de Gasteromycetes en Sonora. *Revista Mexicana de Micología* 6: 91-104.
- Esqueda, M., E. Pérez-Silva, T. Herrera and G. Moreno. 1998. Adiciones al conocimiento de los Gasteromicetos de Sonora, México. *Revista Mexicana de Micología* 14: 41-52.
- Esqueda, M., E. Pérez-Silva, T. Herrera, M. Coronado-Andrade and A. Estrada-Torres. 2000. Composición de Gasteromicetos en



- un gradiente de vegetación de Sonora, México. Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Botánica 71: 39-62.
- Frutis, I. and G. Guzmán. 1983. Contribución al conocimiento de los hongos del estado de Hidalgo. Boletín de la Sociedad Mexicana de Micología 18: 219-266.
- García-Rodríguez, J. L., J. Pérez-Moreno, A. Aldrete, V. M. Cetina-Alcalá and H. Vaquera-Huerta. 2006. Caracterización del hongo silvestre ectomicorrízico *Pisolithus tinctorius* (Pers.) Coker et Couch en cultivo y en simbiosis con Eucalipto y Pino. Agrociencia 40(5): 665-676.
- Gargano, M. L., S. Maisano and G. Venturella. 2018. *Pisolithus albus*, a new record for Italy. Field Mycology 19(3): 86-89. DOI: <https://doi.org/10.1016/j.fldmyc.2018.07.008>
- Garza Ocañas, F., J. García Jiménez, G. Guevara Guerrero, M. Quiñonez Martínez and M. I. Yáñez Díaz. 2023. Especies de macrohongos en matorrales del Noreste de México. Revista Mexicana De Ciencias Forestales 14(79): 213-236. DOI: <https://doi.org/10.29298/rmcf.v14i79.1365>
- Geneious Prime. 2025. Geneious Prime ver. 2025.0.3 <https://www.geneious.com> (consulted January, 2025).
- Gomes, E. A., L. M. Abreu, A. C. Borges and E. F. Araújo. 2000. ITS sequences and mitochondrial DNA polymorphism in *Pisolithus* isolates. Mycology Research 104(8): 911-918. DOI: <https://doi.org/10.1017/S0953756200002586>
- Guzmán, G. and T. Herrera. 1969. Macromicetos de las zonas áridas de México, II. Gasteromicetos. Boletín de la Sociedad Mexicana de Micología 40: 1-92.
- Guzmán, G. and T. Herrera. 1973. Especies de macromicetos citadas de México, IV. Gasteromicetos. Boletín de la Sociedad Mexicana de Micología 7: 105-119.
- Hernández-Navarro, E., M. Esqueda, M. Lizárraga, D. López-Peña and A. Gutiérrez. 2015. Registros nuevos de hongos gasteroides y mixomicetos de la Sierra de Mazatán, Sonora, México. Scientia Fungorum 3(42): 45-52.
- Herrera, T. 1964. Clasificación, descripción y relaciones ecológicas de Gasteromicetos del Valle de México. Anales del Instituto de Biología, Universidad Nacional Autónoma de México 35: 9-43.
- Herrera, T., E. Pérez-Silva and E. Aguirre. 1989. Distribución y hábitat de Sclerodermatales de México. Revista de la Sociedad Mexicana de Historia Natural 40: 59-64.
- Hitchcock, C. J., S. M. Chambers and J. W. Cairney. 2011. Genetic population structure of the ectomycorrhizal fungus *Pisolithus microcarpus* suggests high gene flow in south-eastern Australia. Mycorrhiza 21(2): 131-137. DOI: <https://doi.org/10.1007/s00572-010-0317-3>
- Hosaka, K. 2009. Phylogeography of the genus *Pisolithus* revisited with some additional taxa from New Caledonia and Japan. Bulletin of the National Museum of Nature and Science 35(3): 151-167.
- Huelsenbeck, J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8): 754-755. DOI: <https://doi.org/10.1093/bioinformatics/17.8.754>
- Index Fungorum. 2024. Index Fungorum, database. <http://www.indexfungorum.org/names/Names.asp> (consulted November, 2024).
- Jaouani, A., M. L. Gargano, Z. Ouali, I. Sbissi, R. Compagno and G. Venturella. 2015. *Pisolithus albus* (Sclerodermataceae), a new record for Tunisia. Flora Mediterranea 25: 73-78.
- Jourand, P., M. Ducouso, C. Loulergue-Majorel, L. Hannibal, S. Santoni, Y. Prin and Lebrun, M. 2010. Ultramafic soils from New Caledonia structure *Pisolithus albus* in ecotype. FEMS Microbiology and Ecology 72(2): 238-249. DOI: <http://dx.doi.org/10.1111/j.1574-6941.2010.00843.x>
- Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. von Haeseler and L. S. Jermin. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587-589. DOI: <https://doi.org/10.1038/nmeth.4285>
- Kanchanaprayudh, J., T. Hogetsu, Z. Zhou, S. Yomyart and P. Sihanonth. 2003. Molecular phylogeny of ectomycorrhizal *Pisolithus* fungi associated with pine, dipterocarp, and *Eucalyptus* trees in Thailand. Mycoscience 44(4): 287-294. DOI: <https://doi.org/10.1007/s10267-003-0110-7>
- Kasuya, M. C., I. Coelho, Y. Tamai, T. Miyamoto and T. Yajima. 2008. Morphological and molecular characterization of *Pisolithus* occurring in Hokkaido Island, Northern Japan. Mycoscience 49(5): 334-338. DOI: <https://doi.org/10.1007/S10267-008-0420-X>
- Katoh, K. J. Rozewicki and K. D. Yamada. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160-1166. DOI: <https://doi.org/10.1093/bib/bbx108>



- Lampky, J. R. and J. E. Peterson. 1963. *Pisolithus tinctorius* associated with Pines in Missouri. *Mycologia* 55(5): 675-678. DOI: <https://doi.org/10.2307/3756442>
- Lebel, T., S. Pennycook and M. Barrett. 2018. Two new species of *Pisolithus* (Sclerodermataceae) from Australasia, and an assessment of the confused nomenclature of *P. tinctorius*. *Phytotaxa* 348(3): 163-186. DOI: <https://doi.org/10.11646/phytotaxa.348.3.1>
- Leonard, P. and S. J. McMullan-Fisher. 2013. *Pisolithus* in Queensland. *Fungimap Newsletter* 49: 4-8.
- Leonard, P. L., S. J. McMullan-Fisher and T. Lebel. 2013. *Pisolithus croceorrhizus* P. Leonard & McMullan-Fisher sp. nov. from Queensland, Australia and New Caledonia. *Australasian Mycologist* 31: 25-29.
- Maddison, W. P. and D. R. Maddison. 2023. Mesquite: a modular system for evolutionary analysis, ver. 3.81 <http://www.mesquiteproject.org> (consulted March, 2025).
- Malajczuk, N. and W. Dunstan. 2002. Persistence of some Australian *Pisolithus* species introduced to eucalypt plantations in China. *Forest Ecology and Management* 139(3): 271-281. DOI: [https://doi.org/10.1016/S0378-1127\(01\)00750-2](https://doi.org/10.1016/S0378-1127(01)00750-2)
- Martin, F., C. Delaruelle and M. Ivory. 1998. Genetic variability in intergenic spacers of ribosomal DNA in *Pisolithus* isolates associated with pine, eucalyptus and *Azelia* in lowland Kenyan forests. *New Phytologist* 139(2): 341-352. DOI: <https://doi.org/10.1046/j.1469-8137.1998.00195.x>
- Martin, F., J. Díez, B. Dell and C. Delaruelle. 2002. Phylogeography of the ectomycorrhizal *Pisolithus* species as inferred from nuclear ribosomal DNA ITS sequences. *New Phytologist* 153(2): 345-357. DOI: <https://doi.org/10.1046/j.0028-646X.2001.00313.x>
- Martín, M. P., F. Durán, C. Phosri and R. Watling. 2013. A new species of *Pisolithus* from Spain. *Mycotaxon* 124(1): 149-154. DOI: <https://doi.org/10.5248/124.149>
- Marx, D. H. 1977. Tree host range and world distribution of the ectomycorrhizal fungus *Pisolithus tinctorius*. *Canadian Journal of Microbiology* 23(3): 217-223. DOI: <https://doi.org/10.1139/m77-033>
- Montagner, D. F., G. Coelho, A. O. Silveira, D. B. Baldoni and Z. Antonioli. 2015. Morphological and molecular analyses in *Scleroderma* (Basidiomycota) associated with exotic forests in Pampa biome, southern Brazil. *Mycosphere* 6(3): 337-344. DOI: <https://doi.org/10.5943/mycosphere/6/3/9>
- Moreno, G., M. Lizárraga, M. Esqueda and M. L. Coronado. 2010. Contribution to the study of gasteroid and secotioid fungi of Chihuahua, Mexico. *Mycotaxon* 112(1): 291-315. DOI: <https://doi.org/10.5248/112.291>
- Moyersoen, B., R. E. Beever and F. Martin. 2003. Genetic diversity of *Pisolithus* in New Zealand indicates multiple long-distance dispersal from Australia. *New Phytologist* 160(3): 569-579. DOI: <https://doi.org/10.1046/j.1469-8137.2003.00908.x>
- Nava Mora, R. and R. Valenzuela Garza. 1997. Los macromicetos de la Sierra de Nanchititla, I. *Polibotánica* 5: 21-36.
- NCBI. 2024. National Center for Biotechnology Information (NCBI). National Library of Medicine (US). <https://www.ncbi.nlm.nih.gov> (consulted June-October, 2024).
- Palmer, J. M., D. L. Lindner and T. J. Volk. 2008. Ectomycorrhizal characterization of an American chestnut (*Castanea dentata*)-dominated community in Western Wisconsin. *Mycorrhiza* 19(1): 27-36. DOI: <https://doi.org/10.1007/s00572-008-0200-7>
- Pardavé, L. M. 1991. Gasteromycetes del estado de Aguascalientes. *Revista Mexicana de Micología* 7: 71-78.
- Patel, R. and K. Rajput. 2023. Molecular and morphological studies on some gasteroid (Basidiomycota) fungi from Western India. *Asian Journal of Mycology* 6(2): 72-88. DOI: <https://doi.org/10.5943/ajom/6/2/6>
- Pérez-Silva, E., M. Esqueda and T. Herrera. 1994. Contribución al conocimiento de los Gasteromicetos de Sonora. *Revista Mexicana de Micología* 10: 77-101.
- Phosri, C., M. P. Martín, N. Suwannasai, P. Sihanonth and R. Watling. 2012. *Pisolithus*: a new species from southeast Asia and a new combination. *Mycotaxon* 120(1): 195-208. DOI: <https://doi.org/10.5248/120.195>
- Phosri, C., M. P. Martín, R. Watling, M. Jeppson and P. Sihanonth. 2009. Molecular phylogeny and re-assessment of some *Scleroderma* spp. (Gasteromycetes). *Anales del Jardín Botánico de Madrid* 66(S1): 83-91. DOI: <https://doi.org/10.3989/ajbm.2199>
- Phosri, C., M. P. Martín and R. Watling. 2013. *Astraeus*: hidden dimensions. *IMA Fungus* 4: 347-356. DOI: <https://doi.org/10.5598/imafungus.2013.04.02.13>



- Rambaut, A. 2018. FigTree: tree figure drawing tool version 1.4.4. <http://tree.bio.ed.ac.uk/software/figtree/> (consulted March, 2025).
- Reddy, M. S., S. Singla, K. Natarajan and G. Senthilarasu. 2005. *Pisolithus indicus*, a new species of ectomycorrhizal fungus associated with Dipterocarps in India. *Mycologia* 97(4): 838-843. DOI: <https://doi.org/10.3852/mycologia.97.4.838>
- Rodríguez Alcántar, O., D. Figueroa García and M. de J. Herrera-Fonseca. 2019. Catálogo de los hongos de San Sebastián del Oeste, Jalisco, México. *Acta Botanica Mexicana* 126: e1364. DOI: <https://doi.org/10.21829/abm126.2019.1364>
- Rusevska, K., M. Karadelev, C. Phosri, M. Dueñas, M. T. Telleria, R. Watling and M. P. Martín. 2015. DNA barcoding is an effective tool for differentiating *Pisolithus* species from Macedonia. *Mycotaxon* 130(4): 1007-1016. DOI: <https://doi.org/10.5248/130.1007>
- Sáenz-Hidalgo, H. K., J. L. Jacobo-Cuellar, E. Zúñiga-Rodríguez, G. D. Avila-Quezada, V. Olalde-Portugal, A. Hashem and E. F. Abd_Allah. 2023. Soil Structure and Ectomycorrhizal Root Colonization of Pecan Orchards in Northern Mexico. *Journal of Fungi* 9(4): 440. DOI: <https://doi.org/10.3390/jof9040440>
- Schoch, C. L., K. A. Seifert, S. Huhndorf, V. Robert, J. L. Spouge, C. A. Levesque, W. Chen and Fungal Barcoding Consortium. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* 109(16): 6241-6246. DOI: <https://doi.org/10.1073/pnas.1117018109>
- Sebastiania, M., A. Corrêa, P. Castro and M. Ramos. 2020. *Pisolithus*. In: Amaresan, N., K. Annapurna, A. Sankaranarayanan, M. Senthil Kumar and K. Kumar (eds.). *Beneficial microbes in agro-ecology: bacteria and fungi*. Elsevier. Philadelphia, USA. Pp. 707-726. DOI: <https://doi.org/10.1016/B978-0-12-823414-3.00036-8>
- Singla, S., M. S. Reddy, R. Marmeisse and G. Gay. 2004. Genetic variability and taxonomic position of ectomycorrhizal fungus *Pisolithus* from India. *Microbiological Research* 159(3): 203-210. DOI: <https://doi.org/10.1016/j.micres.2004.03.003>
- Tedersoo, L., V. Mikryukov, S. Anslan, M. Bahram, A. Nasir Khalid, A. Corrales, A. Agan, A. M. Vasco-Palacios, A. Saitta, A. Antonelli, A. C. Rinaldi, A. Verbeken, B. P. Sulistyo, B. Tamgnoue, B. Furneaux, C. Duarte Ritter, C. Nyamukondiwa, C. Sharp, C. Marín, D. Q. Dai, D. Gohar, D. Sharmah, E. Machteld Biersma, E. K. Cameron, E. De Crop, E. Otsing, E. A. Davydov, F. E. Albornoz, F. Q. Brearley, F. Buegger, G. Gates, G. Zahn, G. Bonito, I. Hiiesalu, I. Hiiesalu, I. Zettur, I. C. Barrio, J. Pärn, J. Heilmann-Clausen, J. Ankuda, J. Y. Kupagme, J. Sarapuu, J. G. Maciá-Vicente, J. D. Fovo, J. Geml, J. M. Alatalo, J. Alvarez-Manjarrez, J. Monkai, K. Pöldmaa, K. Runnel, K. Adamson, K. A. Bråthen, K. Pritsch, K. I. Tchan, K. Armolaitis, K. D. Hyde, K. K. Newsham, K. Panksep, L. A. Adebola, L. J. Lamit, M. Saba, M. E. da Silva Cáceres, M. Tuomi, M. Gryzenhout, M. Bauters, M. Bálint, N. Wijayawardene, N. Hagh-Doust, N. S. Yorou, O. Kurina, P. E. Mortimer, P. Meidl, R. Henrik Nilsson, R. Puusepp, R. Casique-Valdés, R. Drenkhan, R. Garibay-Orijel, R. Godoy, S. Alfarraj, S. Rahimlou, S. Pölme, S. V. Dudov, S. Mundra, T. Ahmed, T. Netherway, T. W. Henkel, T. Roslin, V. E. Fedosov, V. G. Onipchenko, W. A. Erandi Yasanthika, Y. Woon Lim, M. Piepenbring, D. Klavina, U. Kõljalg and K. Abarenkov. 2021. The Global Soil Mycobiome consortium dataset for boosting fungal diversity research. *Fungal Diversity* 111: 573-588. DOI: <https://doi.org/10.1007/s13225-021-00493-7>
- Thomas, S. R., W. Dunstan, B. Dell, J. Trappe and N. Malajczuk. 2003. *Pisolithus hypogaeus* sp. nov.: A hypogeous representative of the genus *Pisolithus* from Western Australia. *Mycotaxon* 87: 405-410.
- Trifinopoulos, J., L. T. Nguyen, A. V. Haeseler and B. Q. Minh. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44(W1): W232-W235. DOI: <https://doi.org/10.1093/nar/gkw256>
- Urista, E., J. García and J. Castillo. 1985. Algunas especies de Gasteromycetes del Norte de México. *Revista Mexicana de Micología* 1: 471-523.
- Welden, A. L. and G. Guzmán. 1978. Lista preliminar de los hongos, líquenes y mixomicetos de las regiones de Uxpanapa, Coatzacoalcos, Los Tuxtlas, Papaloapan y Xalapa (parte de los estados de Veracruz y Oaxaca). *Boletín de la Sociedad Mexicana de Micología* 12: 59-102.
- Wickham, H. and W. Chang. 2008. ggplot2: An implementation of the Grammar of Graphics. R package ver. 0.7. <http://CRAN.R-project.org/package=ggplot2> (consulted July, 2025).



Wilson, A. W., M. Binder and D. S. Hibbett. 2011. Effect of gasteroid fruiting body morphology on diversification rate in three independent clades of fungi estimated using binary state speciation and extinction analysis. *Evolution* 65(5): 1305-1322. DOI: <https://doi.org/10.1111/j.1558-5646.2010.01214.x>

Zarco, J. 1986. Estudio de la distribución ecológica de los hongos (principalmente macromicetos) en el Valle de México, basado en los especímenes depositados en el herbario ENCB. *Revista Mexicana de Micología* 2: 41-72.



Appendix 1: List of specimens analyzed. Fungarium collection number, taxonomic ID of the collection according to the herbarium, NCBI (2024) accession, municipality/state, coordinates, date, collector, and potential host. ***Indicates specimen with low quality DNA with no amplification.

Voucher Specimen	Collection ID	ITS Accession Number	Locality	Coordinates	Collection Date	Collectors	Potential Host
MEXU 31772	<i>Pisolithus albus</i> (Cooke & Masee) Priest	PQ380510	Hermosillo, Sonora	29°04'53"N, 110°58'07"W	06/09/1995	M. Esqueda	<i>Eucalyptus</i> sp.
NCBI (2024)	<i>Pisolithus albus</i> (Cooke & Masee) Priest	PQ380509	Caborca, Sonora	30°47'46"N, 112°27'56"W	18/04/2019	R. Gutierrez and A. Gutierrez	<i>Eucalyptus</i> sp.
NCBI (2024)	<i>Pisolithus albus</i> (Cooke & Masee) Priest	PQ380508	Ensenada, Baja California	31°52'12.9"N, 116°39'59.0"W	25/01/2019	E. Hernández-Navarro	<i>Eucalyptus</i> sp.
NCBI (2024)	<i>Pisolithus arhizus</i> (Scop.) Rauschert	PQ380520	Cananea, Sonora	30°59'23.6"N, 110°17'19.9"W	10/09/1993	E. Pérez-Silva and M. Esqueda	<i>Pinus</i> sp.
NCBI (2024)	<i>Pisolithus arhizus</i> (Scop.) Rauschert	***	Acatlán de Pérez Figuroa, Oaxaca	19°02'37.1"N, 100°13'30.2"W	23/06/1976	E. Pérez-Silva et al.	<i>Pinus-Quercus</i>
MEXU 31777	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	***	Yécora, Sonora	28°23'41"N, 109°05'26"W	13/09/1996	M. Esqueda et al.	<i>Pinus</i> sp.
MEXU 31778	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	***	Yécora, Sonora	28°23'41"N, 109°05'26"W	10/09/1995	M. Esqueda et al.	<i>Pinus</i> sp.
MEXU 31676	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380522	Hermosillo, Sonora	28°52'48.8"N, 111°18'14.2"W	03/10/2019	E. Hernández-Navarro	<i>Carya illinoensis</i> (Wangenh.) K. Koch
MEXU 22604	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	***	Yécora, Sonora	28°26'03.8"N, 109°10'34.3"W	05/08/1989	A. Aparicio Navarro	<i>Quercus</i> sp.
MEXU 22605	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	***	Yécora, Sonora	28°22'25.7"N, 108°54'36.7"W	26/07/1989	A. Aparicio Navarro	<i>Quercus</i> sp.
MEXU 1409	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	***	Cuajimalpa de Morelos, CDMX	19°17'51.4"N, 99°18'56.0"W	02/07/1950	T. Herrera	<i>Pinus</i> sp.
MEXU 10485	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	***	Acatlán de Pérez Figuroa, Oaxaca	18°26'44.8"N, 96°25'18.0"W	23/04/1976	E. Pérez-Silva et al.	<i>Pinus-Quercus</i>
MEXU 22336	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380519	San Juan Mixtepec, Oaxaca	17°16'09.6"N, 97°48'07.2"W	03/09/1989	E. Pérez-Silva et al.	<i>Pinus-Quercus</i>
MEXU 21301	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380518	San Juan Mixtepec, Oaxaca	17°18'20.5"N, 97°49'54.7"W	02/10/1988	J. Reyes Santiago	<i>Pinus-Quercus</i>
MEXU 21768	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380528	San Juan Mixtepec, Oaxaca	17°22'52.1"N, 97°51'20.9"W	21/07/1989	J. Reyes Santiago	<i>Pinus pringlei</i> Shaw, <i>Pinus lawsonii</i> Roehl ex Gordon & Glend, <i>Quercus magnoliifolia</i> Née
MEXU 8969	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	***	Tuxtepec, Oaxaca	18°04'20.3"N, 96°08'52.8"W	31/01/1974	R. Singer	<i>Pinus caribaea</i> Morelet
MEXU 31650	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380521	Metztlán, Hidalgo	20°40'30"N, 98°45'09"W	11/10/2023	E. Hernandez-Navarro	<i>Pinus-Quercus</i>
MEXU 13790	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380514	Zimapan, Hidalgo	20°49'08.6"N, 99°15'33.6"W	06/09/1979	R. Hernández	<i>Pinus-Quercus</i>





Appendix 1: Continuation.

Voucher Specimen	Collection ID	ITS Accession Number	Locality	Coordinates	Collection Date	Collectors	Potential Host
MEXU 16021	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380515	Cardonal, Hidalgo	20°38'53.4"N, 98°57'52.5"W	02/10/1980	R. Hernández and D. Rodríguez	-----
MEXU 13200	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	***	Santiago Tulantepec de Lugo Guerrero, Hidalgo	20°03'35.5"N, 98°26'07.3"W	03/07/1979	R. Hernández	<i>Pinus-Quercus</i>
MEXU 9575	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	***	Emiliano Zapata, Veracruz	19°20'29.3"N, 96°30'33.0"W	10/07/1974	R. Hernández	<i>Quercus</i> sp.
MEXU 7876	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380526	Maltrata, Veracruz	18°50'21.2"N, 97°17'08.8"W	28/08/1969	R. Hernandez and R.C. Trigos	<i>Quercus</i> sp.
MEXU 7877	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	***	Maltrata, Veracruz	18°50'21.2"N, 97°17'08.8"W	14/10/1971	R. Hernandez and R.C. Trigos	<i>Quercus</i> sp.
MEXU 17902	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380517	Torreón, Coahuila	25°32'18.9"N, 103°14'13.3"W	12/08/1982	T. Herrera	-----
MEXU 17901	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380516	Saltillo, Coahuila	25°26'56.3"N, 100°57'44.4"W	10/08/1982	T. Herrera	<i>Carya illinoensis</i> (Wangenh.) K. Koch
MEXU 12463	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380513	Ciudad Cuauhtémoc, Chihuahua	28°26'57.8"N, 106°52'10.1"W	25/09/1978	E. Pérez-Silva and R. Hernández	<i>Quercus</i> sp.
MEXU 12005	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380512	Temascaltepec, Mexico State	19°02'37.1"N, 100°13'30.2"W	12/01/1978	R. Hernández	<i>Pinus-Quercus</i>
MEXU 4570	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380525	Tepehuanes, Durango	25°30'10.6"N, 105°36'56.9"W	17/09/1960	F. Sánchez	-----
MEXU 4566	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380523	Chilpancingo de los Bravo, Guerrero	17°17'28.8"N, 99°28'46.0"W	21/06/1963	H. Kruse	<i>Pinus-Quercus</i>
MEXU 4569	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380524	Chilpancingo de los Bravo, Guerrero	17°14'40.5"N, 98°40'30.2"W	13/06/1963	H. Kruse	<i>Pinus-Quercus</i>
MEXU 11637	<i>Pisolithus tinctorius</i> (Mont.) E. Fisch	PQ380527	Tequila, Jalisco	20°40'27.9"N, 103°23'37.1"W	25/08/1977	R. Hernández	<i>Pinus-Quercus</i>
MEXU 11889	<i>Scleroderma albidum</i> Pat. & Trab.	PQ380507	Mineral El Chico, Hidalgo	20°11'46"N 98°43'38"W	12/09/1976	Huacaja et al.	<i>Abies</i> sp.

Appendix 2: List of species, potential host, country of origin, and NCBI (2024) accession number of the downloaded sequences used in the phylogenetic analysis.

Taxa	Accession number	Country of origin	Potential host	Reference
<i>Astraeus hygrometricus</i> (Pers.) Morgan (Neotype)	HG000287	France	-----	Phosri et al., 2013
<i>Pisolithus abditus</i> Kanch., Sihan., Hogetsu & Watling (Isotype)	AB099922	Thailand	<i>Dipterocarpus alatus</i> Roxb. ex G. Don	Kanchanaprayudh et al., 2003
<i>Pisolithus abditus</i> Kanch., Sihan., Hogetsu & Watling	AB099920	Thailand	<i>Anthoshorea roxburghii</i> (G. Don) P.S. Ashton & J. Heck.	Kanchanaprayudh et al., 2003
<i>Pisolithus abditus</i> Kanch., Sihan., Hogetsu & Watling	FR748119	Thailand	<i>Shorea</i> sp.	Phosri et al., 2012
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AF374694	Senegal	<i>Eucalyptus camaldulensis</i> Dehnh.	Martin et al., 2002
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AB099918	Thailand	<i>Eucalyptus camaldulensis</i> Dehnh.	Kanchanaprayudh et al., 2003
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AF228655	Morocco	<i>Eucalyptus</i> sp.	Díez et al., 2001
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AF228656	Spain	<i>Eucalyptus</i> sp.	Díez et al., 2001
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AB099914	Thailand	<i>Eucalyptus camaldulensis</i> Dehnh.	Kanchanaprayudh et al., 2003
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AF228654	Morocco	<i>Eucalyptus</i> sp.	Díez et al., 2001
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AF270782	Australia	<i>Eucalyptus</i> sp.	Anderson et al., 2001
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AF374622	Senegal	<i>Eucalyptus</i> sp.	Martin et al., 2002
<i>Pisolithus albus</i> (Cooke & Masee) Priest	FR748123	Thailand	<i>Eucalyptus camaldulensis</i> Dehnh.	Phosri et al., 2012
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AF374690	Senegal	<i>Eucalyptus</i> sp.	Martin et al., 2002
NCBI (2024)	KY689599	New Zealand	<i>Kunzea</i> sp.	Lebel et al., 2018
NCBI (2024)	KY689592	Australia	<i>Eucalyptus rupestris</i> Brooker & Done	Lebel et al., 2018
NCBI (2024)	AJ629887	Thailand	<i>Eucalyptus camaldulensis</i> Dehnh.	Phosri et al., 2012
NCBI (2024)	FJ874751	Australia	<i>Eucalyptus</i> sp.	Hitchcock et al., 2011
NCBI (2024)	AF374670	Australia	<i>Eucalyptus tereticornis</i> Gaertn., <i>Corymbia tessellaris</i> (F. Muell.) K.D. Hill & L.A.S. Johnson	Martin et al., 2002
<i>Pisolithus albus</i> (Cooke & Masee) Priest	MN295477	Pakistan	-----	Phosri et al., 2012
<i>Pisolithus albus</i> (Cooke & Masee) Priest	FJ874739	Australia	-----	Hitchcock et al., 2011
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AM947069	New Caledonia	<i>Acacia</i> sp.	Jourand et al., 2010
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AM947117	New Caledonia	<i>Acacia spirorbis</i> Labill.	Jourand et al., 2010
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AM947099	New Caledonia	-----	Jourand et al., 2010
<i>Pisolithus albus</i> (Cooke & Masee) Priest	MF510372	India	-----	Patel y Rajput, 2023
<i>Pisolithus albus</i> (Cooke & Masee) Priest	OK184610	Egypt	<i>Eucalyptus occidentalis</i> Endl.	Abdel-Aziz y Bakhit, 2023
<i>Pisolithus albus</i> (Cooke & Masee) Priest	JQ365190	Thailand	-----	Phosri et al., 2012
<i>Pisolithus albus</i> (Cooke & Masee) Priest	AF374688	Senegal	<i>Eucalyptus</i> sp.	Martin et al., 2002
<i>Pisolithus arhizus</i> (Scop.) Rauschert	FR748129	Italy	-----	Phosri et al., 2012





Appendix 2: Continuation.

Taxa	Accession number	Country of origin	Potential host	Reference
<i>Pisolithus arhizus</i> (Scop.) Rauschert	AF228648	Spain	<i>Quercus helferiana</i> A. DC./ <i>Quercus coccifera</i> L.	Díez et al., 2001
<i>Pisolithus arhizus</i> (Scop.) Rauschert	FR748128	Italy	----	Phosri et al., 2012
<i>Pisolithus arhizus</i> (Scop.) Rauschert	FM213365	Spain	<i>Pinus</i> sp.	Phosri et al., 2009
<i>Pisolithus aurantioscabrosus</i> Watling	EU718112	Malasia	<i>Shorea</i> sp.	Phosri et al., 2012
<i>Pisolithus arhizus</i> (Scop.) Rauschert	AF415227	Malasia	<i>Rubroshorea macroptera</i> (Dyer) P.S. Ashton & J. Heck.	Martin et al., 2002
<i>Pisolithus arhizus</i> (Scop.) Rauschert	AF415226	Malasia	<i>Rubroshorea macroptera</i> (Dyer) P.S. Ashton & J. Heck.	Martin et al., 2002
<i>Pisolithus aureosericeus</i> M.P. Martín, Kaewgraj., Phosri & Watling	KU351840	Thailand	<i>Hopea odorata</i> Roxb.	Crous et al., 2016
<i>Pisolithus aureosericeus</i> M.P. Martín, Kaewgraj., Phosri & Watling	KU351838	Thailand	<i>Hopea odorata</i> Roxb.	Crous et al., 2016
<i>Pisolithus aureosericeus</i> M.P. Martín, Kaewgraj., Phosri & Watling	KU351839	Thailand	<i>Hopea odorata</i> Roxb.	Crous et al., 2016
<i>Pisolithus aureosericeus</i> M.P. Martín, Kaewgraj., Phosri & Watling (Isotype)	KU351837	Thailand	<i>Hopea odorata</i> Roxb.	Crous et al., 2016
<i>Pisolithus calongei</i> M.P. Martín, Phosri & Watling	FR748140	Spain	<i>Cistus</i> sp.	Phosri et al., 2012
<i>Pisolithus calongei</i> M.P. Martín, Phosri & Watling	AF228644	Spain	<i>Cistus ladanifer</i> L.	Díez et al., 2001
<i>Pisolithus calongei</i> M.P. Martín, Phosri & Watling	AF228642	Spain	<i>Cistus ladanifer</i> L.	Díez et al., 2001
<i>Pisolithus calongei</i> M.P. Martín, Phosri & Watling	AF228641	Spain	<i>Cistus ladanifer</i> L.	Díez et al., 2001
<i>Pisolithus calongei</i> M.P. Martín, Phosri & Watling (Holotype)	HE578141	Spain	<i>Cistus ladanifer</i> L.	Martin et al., 2013
<i>Pisolithus capsulifer</i> (Sowerby) Watling, Phosri & M.P. Martín	AF374629	Japan	<i>Pinus pumila</i> (Pall.) Regel	Martin et al., 2002
<i>Pisolithus capsulifer</i> (Sowerby) Watling, Phosri & M.P. Martín	FR748134	England	----	Phosri et al., 2012
<i>Pisolithus capsulifer</i> (Sowerby) Watling, Phosri & M.P. Martín (Epitype)	FR748135	England	----	Phosri et al., 2012
<i>Pisolithus capsulifer</i> (Sowerby) Watling, Phosri & M.P. Martín	FR748136	England	----	Phosri et al., 2012
<i>Pisolithus croceorrhizus</i> P. Leonard & McMull.-Fish. (Holotype)	JX444161	Australia	----	Lebel et al., 2018
<i>Pisolithus croceorrhizus</i> P. Leonard & McMull.-Fish.	KY689612	Australia	<i>Acacia</i> sp., <i>Eucalyptus</i> sp.	Lebel et al., 2018
<i>Pisolithus croceorrhizus</i> P. Leonard & McMull.-Fish.	KY689610	Australia	<i>Eucalyptus miniata</i> A. Cunn. ex Schauer	Lebel et al., 2018
<i>Pisolithus hypogaeus</i> S.R. Thomas, Dell & Trappe (Holotype)	AY179747	Australia	<i>Eucalyptus</i> sp.	Thomas et al., 2003
<i>Pisolithus hypogaeus</i> S.R. Thomas, Dell & Trappe	AY179746	Australia	<i>Eucalyptus</i> sp.	Thomas et al., 2003
<i>Pisolithus indicus</i> Natarajan & Senthil (Holotype)	AY756113	India	<i>Vateria indica</i> L.	Reddy et al., 2005

Appendix 2: Continuation.

Taxa	Accession number	Country of origin	Potential host	Reference
<i>Pisolithus madagascariensis</i> Rivas-Ferreiro, Dentinger, Suz & A.M. Ainsw. (Isotype)	OR704320	Madagascar	-----	Crous et al., 2024
<i>Pisolithus madagascariensis</i> Rivas-Ferreiro, Dentinger, Suz & A.M. Ainsw. (Holotype)	OR704319	Madagascar	-----	Crous et al., 2024
<i>Pisolithus marmoratus</i> (Berk.) E. Fisch.	KY689589	Australia	<i>Eucalyptus miniata</i> A. Cunn. ex Schauer, <i>E. tetradonta</i> F. Muell.	Lebel et al., 2018
<i>Pisolithus marmoratus</i> (Berk.) E. Fisch.	AF440866	China	<i>Eucalyptus</i> sp.	Malajczuk y Dunstan, 2002
<i>Pisolithus marmoratus</i> (Berk.) E. Fisch.	AF374719	Australia	<i>Corymbia calophylla</i> (Lindl.) K.D. Hill & L.A.S. Johnson	Martin et al., 2002
<i>Pisolithus marmoratus</i> (Berk.) E. Fisch.	AF374644	Australia	<i>Eucalyptus globulus</i> Labill.	Martin et al., 2002
<i>Pisolithus marmoratus</i> (Berk.) E. Fisch.	AF003914	Kenya	<i>Eucalyptus camaldulensis</i> Dehnh.	Díez et al., 2001
<i>Pisolithus marmoratus</i> (Berk.) E. Fisch.	AF004734	Wales	<i>Eucalyptus</i> sp.	Anderson et al., 1998
<i>Pisolithus marmoratus</i> (Berk.) E. Fisch.	AF004733	Wales	<i>Eucalyptus</i> sp.	Anderson et al., 1998
<i>Pisolithus marmoratus</i> (Berk.) E. Fisch.	HQ693099	Brazil	<i>Eucalyptus</i> sp.	Kasuya et al., 2008
<i>Pisolithus microcarpus</i> (Cooke & Masee) G. Cunn.	AF004735	Wales	<i>Eucalyptus</i> sp.	Anderson et al., 1998
<i>Pisolithus microcarpus</i> (Cooke & Masee) G. Cunn.	AF142991	Brazil	<i>Eucalyptus</i> sp.	Gomes et al., 2000
<i>Pisolithus microcarpus</i> (Cooke & Masee) G. Cunn.	AF228657	Morocco	<i>Eucalyptus</i> sp.	Díez et al., 2001
<i>Pisolithus microcarpus</i> (Cooke & Masee) G. Cunn.	AF374704	Brazil	<i>Eucalyptus dunni</i> Maiden	Martin et al., 2002
<i>Pisolithus microcarpus</i> (Cooke & Masee) G. Cunn.	AF270784	Wales	<i>Eucalyptus</i> sp.	Anderson et al., 2001
<i>Pisolithus microcarpus</i> (Cooke & Masee) G. Cunn.	AF374718	Australia	<i>Eucalyptus</i> sp.	Martin et al., 2002
<i>Pisolithus microcarpus</i> (Cooke & Masee) G. Cunn.	AF374674	Australia	<i>Eucalyptus</i> sp.	Martin et al., 2002
<i>Pisolithus orientalis</i> Watling, Phosri & M.P. Martín	AB099919	Thailand	<i>Pinus kesiya</i> Royle ex Gordon	Martin et al., 2002
<i>Pisolithus orientalis</i> Watling, Phosri & M.P. Martín	AF374711	China	<i>Eucalyptus</i> sp./ <i>Pinus</i> sp.	Martin et al., 2002
<i>Pisolithus orientalis</i> Watling, Phosri & M.P. Martín	FR748149	Thailand	<i>Pinus kesiya</i> Royle ex Gordon	Phosri et al., 2012
<i>Pisolithus orientalis</i> Watling, Phosri & M.P. Martín	AF374679	China	<i>Eucalyptus</i> sp.	Martin et al., 2002
<i>Pisolithus orientalis</i> Watling, Phosri & M.P. Martín	AB099845	Thailand	<i>Pinus kesiya</i> Royle ex Gordon	Kanchanaprayudh et al., 2003
<i>Pisolithus orientalis</i> Watling, Phosri & M.P. Martín	AF374625	Thailand	<i>Pinus kesiya</i> Royle ex Gordon	Martin et al., 2002
<i>Pisolithus orientalis</i> Watling, Phosri & M.P. Martín (Holotype)	FR748148	Thailand	<i>Pinus kesiya</i> Royle ex Gordon	Phosri et al., 2012
<i>Pisolithus thermaeus</i> T. Lebel, Pennycook & Beever	KY689621	New Zealand	<i>Kunzea</i> sp.	Lebel et al., 2018
<i>Pisolithus thermaeus</i> T. Lebel, Pennycook & Beever	KY689625	New Zealand	<i>Kunzea</i> sp.	Lebel et al., 2018
<i>Pisolithus thermaeus</i> T. Lebel, Pennycook & Beever (Holotype)	KY689622	New Zealand	<i>Kunzea</i> sp.	Lebel et al., 2018





Appendix 2: Continuation.

Taxa	Accession number	Country of origin	Potential host	Reference
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF003916	Kenya	<i>Pinus caribaea</i> Morelet	Díez et al., 2001
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF374712	France	<i>Pinus</i> sp.	Martin et al., 2002
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF374626	Portugal	<i>Pinus pinaster</i> Aiton	Martin et al., 2002
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch. (Holotype)	AF374632	USA	<i>Pinus elliottii</i> Engelm.	Martin et al., 2002
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF374630	Portugal	<i>Pinus pinaster</i> Aiton/ <i>Quercus suber</i> L.	Martin et al., 2002
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF374634	USA	<i>Pinus</i> sp.	Martin et al., 2002
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF143234	France	----	Gomes et al., 2000
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF228645	Spain	<i>Quercus ilex</i> L./ <i>Quercus coccifera</i> L.	Díez et al., 2001
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF374636	Portugal	<i>Pinus pinaster</i> Aiton/ <i>Quercus suber</i> L.	Martin et al., 2002
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF374633	Nicaragua	<i>Pinus caribaea</i> Morelet	Martin et al., 2002
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	EU718114	USA	----	Wilson et al., 2011
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF228646	Spain	<i>Quercus ilex</i> L./ <i>Cistus ladanifer</i> L.	Díez et al., 2001
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF374707	France	<i>Pinus pinaster</i> Aiton	Martin et al., 2002
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	KC146359	USA	----	Martin et al., 2002
<i>Pisolithus tinctorius</i> (Mont.) E. Fisch.	AF228647	Kenya	<i>Pinus caribaea</i> Morelet	Díez et al., 2001
<i>Pisolithus tympanobaculus</i> T. Lebel & M.D. Barrett	AF374648	Australia	<i>Eucalyptus</i> sp.	Martin et al., 2002
<i>Pisolithus tympanobaculus</i> T. Lebel & M.D. Barrett	AF374658	Australia	<i>Eucalyptus</i> sp.	Martin et al., 2002
<i>Pisolithus tympanobaculus</i> T. Lebel & M.D. Barrett	AF374646	Australia	<i>Eucalyptus</i> sp.	Martin et al., 2002
<i>Pisolithus tympanobaculus</i> T. Lebel & M.D. Barrett (Isotype)	KY689619	Australia	<i>Eucalyptus</i> sp.	Lebel et al., 2018
<i>Scleroderma albidum</i> Pat. & Trab.	KJ676525	Brazil	----	Montagner et al., 2015
<i>Scleroderma cepa</i> Pers.	EU819439	USA	<i>Castanea dentata</i> (Marshall) Borkh.	Palmer et al., 2008
<i>Scleroderma citrinum</i> Pers.	FM213344	USA	----	Phosri et al., 2009
<i>Scleroderma citrinum</i> Pers.	FM213345	USA	----	Phosri et al., 2009