



Discovery of *Pleurotus cystidiosus* (Agaricales, Pleurotaceae) in the north of Mexico

Hallazgo de *Pleurotus cystidiosus* (Agaricales, Pleurotaceae) en el norte de México

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Abstract:

Background and Aims: Some species of *Pleurotus* form anamorphs that produce coremia and arthrospores, which are classified in the subgenus *Coremiopleurotus* and are morphologically very similar. In Mexico, seven species of the genus are known; however, only *P. smithii*, described from Mexico City, is known to form coremia. Recent collections of *Pleurotus* from Chihuahua, Mexico, showed distinct characteristics from the known species for the country, whose morphological and molecular characterization allowed us to identify *P. cystidiosus* in Mexico. The objective of this work was to document the presence of *P. cystidiosus* in Mexico and provide a detailed description of the specimens found in the country.

Methods: The material was collected in Ciudad Juárez, Chihuahua, Mexico. Specimens were characterized macro- and microscopically through *in situ* photographs, polyfocal, and light microscopy. Morphological identification was performed using specialized literature. DNA extraction was carried out following a 3% CTAB protocol. The ITS region was amplified using the primer pairs ITS5/ITS4. Phylogenetic analyses were conducted using Maximum Likelihood and Bayesian Inference.

Key results: *Pleurotus cystidiosus*, a species previously unreported in Mexico, is morphologically and taxonomically described, confirmed with barcode sequences. The species is distinguished by the presence of abundant clavate to pyriform pleurocystidia and subglobose cheilocystidia in the teleomorph, as well as the formation of coremia with a white stipe, black head, and segments of arthrospores up to 17-25 × 5-7.5 μm in the anamorph.

Conclusions: The presence of *P. cystidiosus* in the north of Mexico is confirmed. With this study, the number of known *Pleurotus* species for Mexico increases to eight.

Key words: arid zones, Basidiomycota, coremia, drylands fungi, oyster fungus.

Resumen:

Antecedentes y Objetivos: Algunas especies de *Pleurotus* forman anamorfos que producen coremios y artrosporas, las cuales están clasificadas en el subgénero *Coremiopleurotus* y son muy similares morfológicamente. En México, se conocen siete especies del género; sin embargo, solo *P. smithii*, descrita de la Ciudad de México, es conocida por formar coremios. Colectas recientes de *Pleurotus* de Chihuahua, México, mostraron características distintas de las especies conocidas para el país, cuya caracterización morfológica y molecular nos permitió identificar a *P. cystidiosus* en México. El objetivo de este trabajo fue documentar la presencia de *P. cystidiosus* en México y brindar una descripción detallada de los ejemplares encontrados en el país.

Métodos: El material fue recolectado en Ciudad Juárez, Chihuahua, México. Los ejemplares fueron caracterizados macro y microscópicamente mediante fotografías *in situ*, microscopía polifocal y de luz. La identificación morfológica se realizó mediante literatura especializada. La extracción de ADN se realizó siguiendo un protocolo de CTAB al 3%. La región de ITS fue amplificada con los pares de cebadores ITS5/ITS4. Los análisis filogenéticos fueron llevados a cabo por Máxima Verosimilitud e Inferencia Bayesiana.

Resultados clave: *Pleurotus cystidiosus*, una especie que no había sido previamente reportada en México, es descrita morfológica y taxonómicamente, confirmada con secuencias de código de barras. La especie se distingue por la presencia de abundantes pleurocistidios clavados a piriformes, y queilocistidios subglobosos en el teleomorfo, así como por la formación de coremios con estípote blanco, cabeza negra y segmentos de artrosporas de hasta 17-25 × 5-7.5 μm en el anamorfo.

Conclusiones: Se confirma la presencia de *P. cystidiosus* en el norte de México. Con este estudio aumenta a ocho el número de especies de *Pleurotus* conocidas para México.

Palabras clave: Basidiomycota, coremios, hongos de zonas secas, hongo ostra, zonas áridas.

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Received: February 13, 2024.

Reviewed: May 7, 2024.

Accepted by Víctor Bandala Muñoz: June 10, 2024.

Published Online first: July 2, 2024.

Published: Acta Botanica Mexicana 131(2024).

To cite as: Hernández-Navarro, E., D. López-Peña and M. Lizárraga-Escobar. 2024. Discovery of *Pleurotus cystidiosus* (Agaricales, Pleurotaceae) in the north of Mexico. Acta Botanica Mexicana 131(2024): e2326. DOI: <https://doi.org/10.21829/abm131.2024.2326>



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e-ISSN: 2448-7589

Introduction

Pleurotus (Fr.) P. Kumm. (Agaricales, Pleurotaceae) is a genus of saprophytic fungi of great importance since most of its species are edible, and some are considered medicinal (Lin et al., 2022). Several species can be cultured on diverse agricultural and industrial waste materials, which has led to the cultivation of several species of *Pleurotus* representing a viable source of income through the production of low-cost food with high quality (Gregory et al., 2007; Raman et al., 2021). Species in this genus are characterized by the flabelliform to dimidiate pileus, attached to the substrate by a short, lateral, sometimes eccentric stipe, which occasionally is absent; gills are decurrent, tight or widely separated, with margin smooth to crenate and involute; hyphal system can be monomitic or dimitic and the spore print is whitish, cream color, or lilac (Flecha Rivas et al., 2014).

Pleurotus species could be confused with other genera such as *Hohenbuehelia* S. Schulz., which has rubbery and flexible fruiting bodies and thick-walled sterile cells on the gills; *Panus* Fr. and *Panellus* P. Karst. characterized by their solid fruiting bodies with a hairy cap surface, and amyloid spores; and *Phyllotopsis* E.-J. Gilbert & Donk ex Singer, which has pinkish allantoid spores (Arora, 1986). Furthermore, *Lentinus* Fr. can also be confused with some of the previous genera; nevertheless, it differs due to its elastic fruiting bodies when young, becoming leathery at maturity, and gills typically with serrated edges (Moreno, 1975).

Due to these problems with the determination of the species and the taxonomic relationships with similar genera, until the year 2000, 23 species and varieties of *Pleurotus* were reported in Mexico. The taxonomic revision of Guzmán (2000) reduced from the 23 species to only seven. From those, two are now accepted as *Lentinus* species (Index Fungorum, 2023): *L. scleropus* (Pers.) Fr. (= *P. hirtus* Guzmán) and *L. levis* (Berk. & M.A. Curtis) Murrill (= *P. levis* (Berk. & M.A. Curtis) Singer). Then, the five known *Pleurotus* species in Mexico, recognized by Guzmán (2000) are *P. bajacalifornicus* Esteve-Rav., G. Moreno & N. Ayala (Moreno et al., 1993); *P. djamor* var. *djamor* (Rumph. ex Fr.) Boedijn (Guzmán et al., 1993); *P. opuntiae* (Durieu & Lév.) Sacc. (Estrada-Torres and Aroche, 1987); *P. ostreatus* (Jacq.)

P. Kumm. (Guzmán, 1977) and *P. smithii* Guzmán (Guzmán, 1975). In addition, *P. dryinus* (Pers.: Fr.) Kumm. was reported by Guzmán (1975) and Valenzuela et al. (1981), but Guzmán (2000) considered it as a non-recognized species in “a complex species in Mexico”. Moreno-Fuentes et al. (1994) also reported *P. dryinus* from Chihuahua, the same state where later Moreno-Fuentes et al. (2004) recorded *P. floridanus* Singer, thus resulting in a total of seven species of *Pleurotus* in Mexico.

Some species of *Pleurotus* are known to produce anamorphs with arthrospores on coremia (= synnemata) (Hilber, 1982). Such anamorphs were previously placed within the genus *Antromycopsis* Pat. & Trab. and they were classified as Hyphomycetes (Deuteromycotina) (Stalpers et al., 1991). However, Camino-Vilaró and Mena-Portales (2019) proposed to conserve the species under the name *Pleurotus cystidiosus* O.K. Mill. Hilber (1982) grouped species of *Pleurotus* with coremia formation under the subgenus *Coremiopleurotus* Hilber (Capelari and Fungaro, 2003) and assigned *P. cystidiosus* (1969) as its type species. Other species with coremia formation are *P. abalonus* Y.H. Han, K.M. Chen & S. Cheng; *P. australis* Cooke & Masee; *P. fuscosquamulosus* Reid & Eicker; *P. purpureo-olivaceus* Segedin, P.K. Buchanan & J.P. Wilkie; and *P. smithii* (Guzmán et al., 1980; Capelari and Fungaro, 2003; Lin et al., 2022). This latter, described from Mexico City, is the only member of *Coremiopleurotus* known from Mexico. In recent sampling carried out by the authors of the present work, some *Pleurotus* specimens with the formation of coremia were observed and collected in Ciudad Juárez, Chihuahua, Mexico, whose morphological and molecular characterization led them to report them as *P. cystidiosus* as a new record for Mexico. Hence, the objective of this work is to document the presence of *P. cystidiosus* in Mexico and provide a detailed description of the specimens found in the country.

Materials and Methods

Fungal material, isolation, and morphological characterization

Basidiomata were observed on an *Ulmus* sp. tree in the urban zone of Ciudad Juárez, in Chihuahua State, Mexico



(31°44'22.6"N; 106°26'33.3"W) (Fig. 1) and collected in September 2021 and August 2022. To isolate a strain, portions of context from the fruiting bodies were inoculated in Petri dishes with potato dextrose agar (PDA) in aseptic conditions and maintained in the dark at 25±1 °C until full colonization. The shape, color, and size of basidiomata were recorded in fresh specimens. Microscopical analyses were carried out by manual cuts of different parts of the basidiomata and coremia, mounted in 5% KOH and observed with a Nikon C-DS stereo microscope (Tokyo, Japan) and a Nikon Eclipse E200 microscope (Tokyo, Japan), equipped with a Nikon CoolPix 4500 camera (Tokyo, Japan). The coremia grown in Petri dishes were photographed using a polyfocal microscope Leica Z16 APO A (Wetzlar, Germany) and processed in the Leica Application Suite v. 4.3 software (Wetzlar, Germany).

The specimens studied are deposited in the herbarium MEXU (Thiers, 2024), with duplicates in the UACJ herbarium of the Universidad Autónoma de Ciudad Juárez, Mexico.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was isolated from a small sample of mycelium that was cultivated on a PDA medium following a CTAB 3% extraction protocol (Doyle and Doyle, 1987). The mycelium was placed in a 2 ml tube with a sterilized Tungsten Sphere, frozen in liquid nitrogen, and ground with a QIAGEN TissueLyser LT (Hilden, Germany). After adding 500 µl of CTAB and 2 µl of β-mercaptoethanol per sample, the tubes were incubated at 45 °C for 30 minutes at 300 rpm in an Eppendorf Thermomixer C (Hamburg, Germany). Next, 500 µl of SEVAG (chloroform: isoamyl alcohol; 24:1) was added and

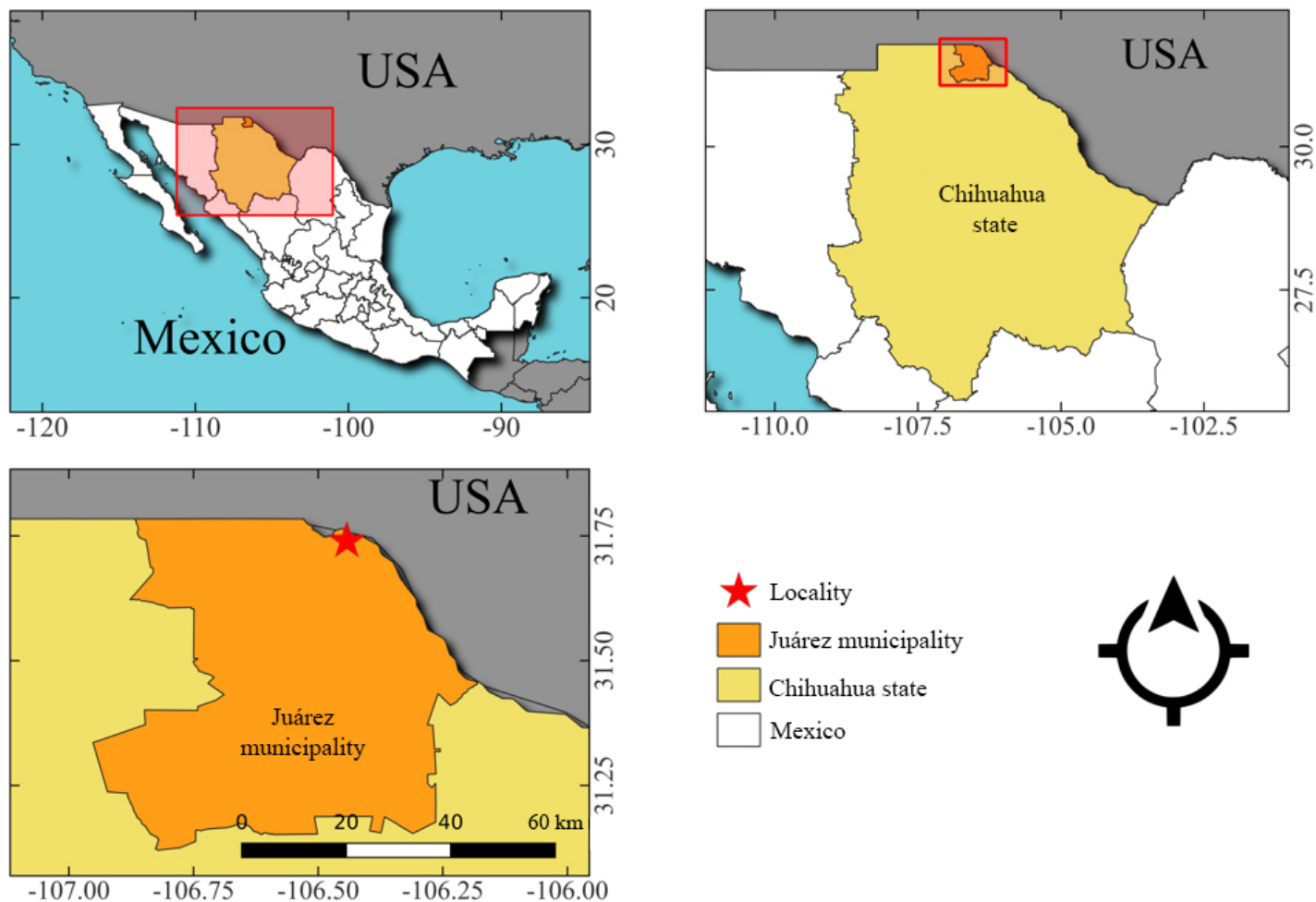


Figure 1: Collection location of *Pleurotus cystidiosus* O.K. Miller, specimens in Chihuahua, Mexico.



stirred for 30 minutes at 85 rpm in a Fisher Clinical Rotator (Hampton, USA). The mixture was centrifuged at 13000 × g for 10 minutes in an Eppendorf centrifuge 5424R (Hamburg, Germany). The supernatant was transferred to a 1.5 ml tube, and 500 µl of isopropanol was added, gently mixed, and stored at -20 °C for one hour. Subsequently, the mixture was centrifuged for 10 minutes at 12000 × g, in a Eppendorf centrifuge 5424R (Hamburg, Germany) and the supernatant was discarded. The pellet was washed with 70% EtOH at -20 °C, vacuum-dried for 5 minutes, and suspended in 50 µl of ultrapure water. The sample was quantified using a NanoDrop 2000 instrument (Waltham, USA), and its integrity was visually verified on a 1% agarose gel stained with RedGel™ using an Analytik Jena UVP transilluminator (Jena, Germany).

The ITS5 (5'-TCCTCCGCTATTGATATGC-3') and ITS4 (5'-GGAAGTAAAAGTCGTAACAAGG-3') were used to amplify the complete ITS1-5.8S-ITS2 region (Schoch et al., 2012) using the PCR Mix (5'BIO, Mexico) and a Thermofisher Scientific Veriti Thermocycler (Waltham, USA). The PCR amplicons were visualized on a 1% agarose gel stained with RedGel™ and the successful amplicons underwent treatment with ExoSAP-IT™ according to the instructions provided by the manufacturer. Clean PCR reactions were sequenced at both ends in the Laboratorio de Secuenciación Genómica de la Biodiversidad of the Laboratorio Nacional de Biodiversidad (LANABIO) of the Instituto de Biología, Universidad Nacional Autónoma de México.

Phylogenetic analyses

The obtained sequence was assembled and curated by inspecting their chromatograms in Sequencher software v. 5.2.3 (Ann Arbor, USA). Reference sequences from specialized literature (Zervakis et al., 2004; 2019; Yang et al., 2007; Horisawa et al., 2013; Liu et al., 2015; Shnyreva and Shnyreva, 2015; Barh et al., 2019; Lin et al., 2022) were downloaded from the National Center for Biotechnology Information (GenBank, 2024) (Table 1), and aligned using the online version of MAFFT v. 7 (Katoh et al., 2002, 2017; Katoh and Standley, 2013).

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. The matrices consisted of 51 sequences of ITS and

included 712 positions. Phylogenetic inferences were estimated using the Maximum Likelihood (ML) method in RAxML v. 8.2.10 (Stamatakis, 2014) with a GTR+G nucleotide substitution model with 1000 bootstrap resampling replicates performed with the GTR+G model.

Bayesian analysis was conducted using Mr Bayes 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

We successfully amplified and sequenced the ITS region of the *Pleurotus* collection from Chihuahua (GenBank accession of the ITS sequence: OR532762). The initial BLAST yielded similarity (>99%) with *P. cystidiosus* from voucher specimens and isolate strains. Both Bayesian and Maximum Likelihood analyses (Fig. 2) grouped our sequence as *P. cystidiosus*, supporting the existence of this distinctive taxon in the north of our country (Bayesian posterior probability (PP) 1 98% and bootstrap resampling values for ML 98%).

Taxonomy

Basidiomycota

Agaricomycetes

Agaricales

Pleurotaceae

Pleurotus cystidiosus O.K. Mill. Mycologia 61: 889, 1969.
Figs. 3, 4.

= *Stilbum macrocarpum* Ellis & Everh., J. Mycol. 2(9): 103. 1886. (Ellis & Everh.) Stalpers, Seifert & Samson, Can. J. Bot. 69(1): 7. 1991.



Table 1: Species, vouchers, localities, and GenBank (2024) accessions of the *Pleurotus* (Fr.) P. Kumm. specimens for the phylogenetic analysis. The *Agaricus* and *Psathyrella* accessions were used as outgroups. The sequence obtained in this study is marked in bold.

Taxon	Voucher/Strain	Locality	ITS Accession Number
<i>Agaricus bisporus</i> (J.E. Lange) Imbach	CBS 11668	USA	MH859080
<i>Pleurotus abalonus</i> Y.H. Han, K.M. Chen & S. Cheng	A1	China	JN671965
<i>Pleurotus abalonus</i> Y.H. Han, K.M. Chen & S. Cheng	149	China	KX688474
<i>Pleurotus abalonus</i> Y.H. Han, K.M. Chen & S. Cheng	ATCC 28787	Taiwan	AY315798
<i>Pleurotus abalonus</i> Y.H. Han, K.M. Chen & S. Cheng	CBS 100129	China	NR103594
<i>Pleurotus abalonus</i> Y.H. Han, K.M. Chen & S. Cheng	CGMCC 37470	China	KX787086
<i>Pleurotus abieticola</i> R.H. Petersen & K.W. Hughes	3509	China	MK209085
<i>Pleurotus abieticola</i> R.H. Petersen & K.W. Hughes	HMJAU56580	China	MT163335
<i>Pleurotus abieticola</i> R.H. Petersen & K.W. Hughes	HKAS45570	China	KP771697
<i>Pleurotus abieticola</i> R.H. Petersen & K.W. Hughes	HKAS45720	China	KP771696
<i>Pleurotus abieticola</i> R.H. Petersen & K.W. Hughes	HKAS46100	China	KP771695
<i>Pleurotus australis</i> Sacc.	CBS 100127	China	EU424276
<i>Pleurotus cystidiosus</i> O.K. Mill.	CBS 29735	USA	AY315766
<i>Pleurotus cystidiosus</i> O.K. Mill.	FP1683	USA	AY315776
<i>Pleurotus cystidiosus</i> O.K. Mill.	D412, D413, D417, D419, D420	USA	AY315774, AY315770, AY315773, AY315771, AY315767
<i>Pleurotus cystidiosus</i> O.K. Mill.	VT1780	USA	AY315769
<i>Pleurotus cystidiosus</i> O.K. Mill.	CCBAS 466	USA	FJ608592
<i>Pleurotus cystidiosus</i> O.K. Mill.	AG 55/466 CCBAS	Russia	FJ608592
<i>Pleurotus cystidiosus</i> O.K. Mill.	P19, P25	China	EF437221 KY962441
<i>Pleurotus cystidiosus</i> O.K. Mill.	ATCC28597	China	EF514244
<i>Pleurotus cystidiosus</i> O.K. Mill.	MEXU 30551	Mexico	OR532762
<i>Pleurotus cystidiosus</i> O.K. Mill.	IFO30784	Korea	AY265818
<i>Pleurotus cystidiosus</i> O.K. Mill.	NBRC 30607	Japan	AB733141
<i>Pleurotus cystidiosus</i> O.K. Mill.	ATCC 28598	South Africa	AY315777
<i>Pleurotus dryinus</i> (Pers.) P. Kumm.	AG I/467, AG II/468, AG III/470	Russia	KF932722, KF932723, KF932724
<i>Pleurotus dryinus</i> (Pers.) P. Kumm.	TENN 7947	Denmark	AY450343
<i>Pleurotus dryinus</i> (Pers.) P. Kumm.	P45, P99	China	KY962461, MG282439
<i>Pleurotus fuscusquamulosus</i> D.A. Reid & Eicker	EGDA PI23	Egypt	MW915606
<i>Pleurotus fuscusquamulosus</i> D.A. Reid & Eicker	LGAM P50	Grece	KF280330
<i>Pleurotus salmoneostramineus</i> Lj.N. Vassiljeva	P36	China	KY962452
<i>Pleurotus salmoneostramineus</i> Lj.N. Vassiljeva	YL1	China	AY728273
<i>Pleurotus smithii</i> Guzmán	CCRC 36229, CBS 68982	Mexico	AY265851
<i>Pleurotus smithii</i> Guzmán	CBS 68082	Mexico	EU424317
<i>Pleurotus smithii</i> Guzmán	ATCC 46391	Mexico	AY315784
<i>Pleurotus tuber-regium</i> (Fr.) Singer	MCCT P7	India	OQ916341
<i>Pleurotus tuber-regium</i> (Fr.) Singer	PYKM100	India	OQ891321
<i>Pleurotus tuber-regium</i> (Fr.) Singer	MCCT AP2	India	OQ920982
<i>Psathyrella secotioides</i> G. Moreno, Heykoop, Esqueda & Olariaga	AH31746	Mexico	KR003281



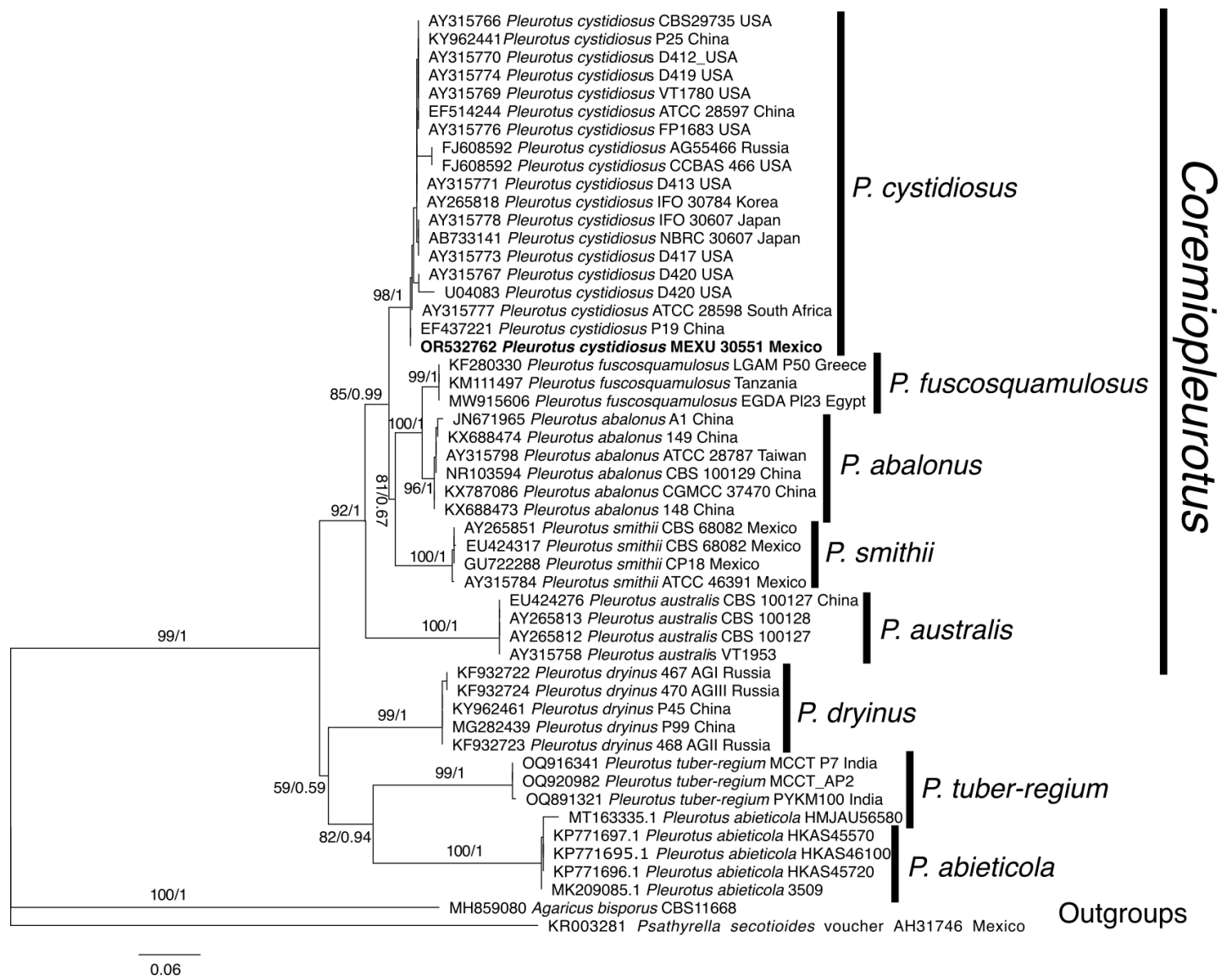


Figure 2: Phylogram of Bayesian inference (BI) tree from the ITS sequence data of 50 specimens. The numbers above branches represent Bootstrap Values (BS) for Maximum Likelihood and Bayesian Posterior Probabilities (PP), respectively. The scale bar represents the expected number of nucleotide substitutions per site. The sequence obtained in this study is in bold. Accession numbers of GenBank (2024) are indicated in each sequence.

= *Antromycopsis broussonetiae* Pat. & Trab., Bull. Soc. Mycol. Fr. 13: 215. 1897.

HOLOTYPE: USA. Indiana, Brown County, Hoosier National Forest, *F.H. Berry CS-66-083-2-A* (BFDL).

Teleomorph

Basidiomata pileate, substipitate to sessile, growing in overlapping groups of up to 15 individuals; pileus plane-convex, flabelliform, 60-130 mm in diameter, surface smooth to

wrinkled, whitish when young, turning yellowish-cream to buff color when mature; lamellae decurrent, tight, white when young, turning yellowish-cream when mature; pseudostipe lateral, 5-10 mm long, eccentric; basidiospores $14-17.5 \times 4-5 \mu\text{m}$, cylindrical, hyaline, inamyloid, thin-walled; basidia $40-65 \times 7-10 \mu\text{m}$, clavate; pleurocystidia $40-50 \times 7-8 \mu\text{m}$, cylindrical, hyaline to light brown, with abundant granular content; cheilocystidia $20-28 \times 8-10 \mu\text{m}$, subglobose to pyriform, thin-walled, hyaline to ochraceous; hyphal system monomitic; pileus trama formed by genera-

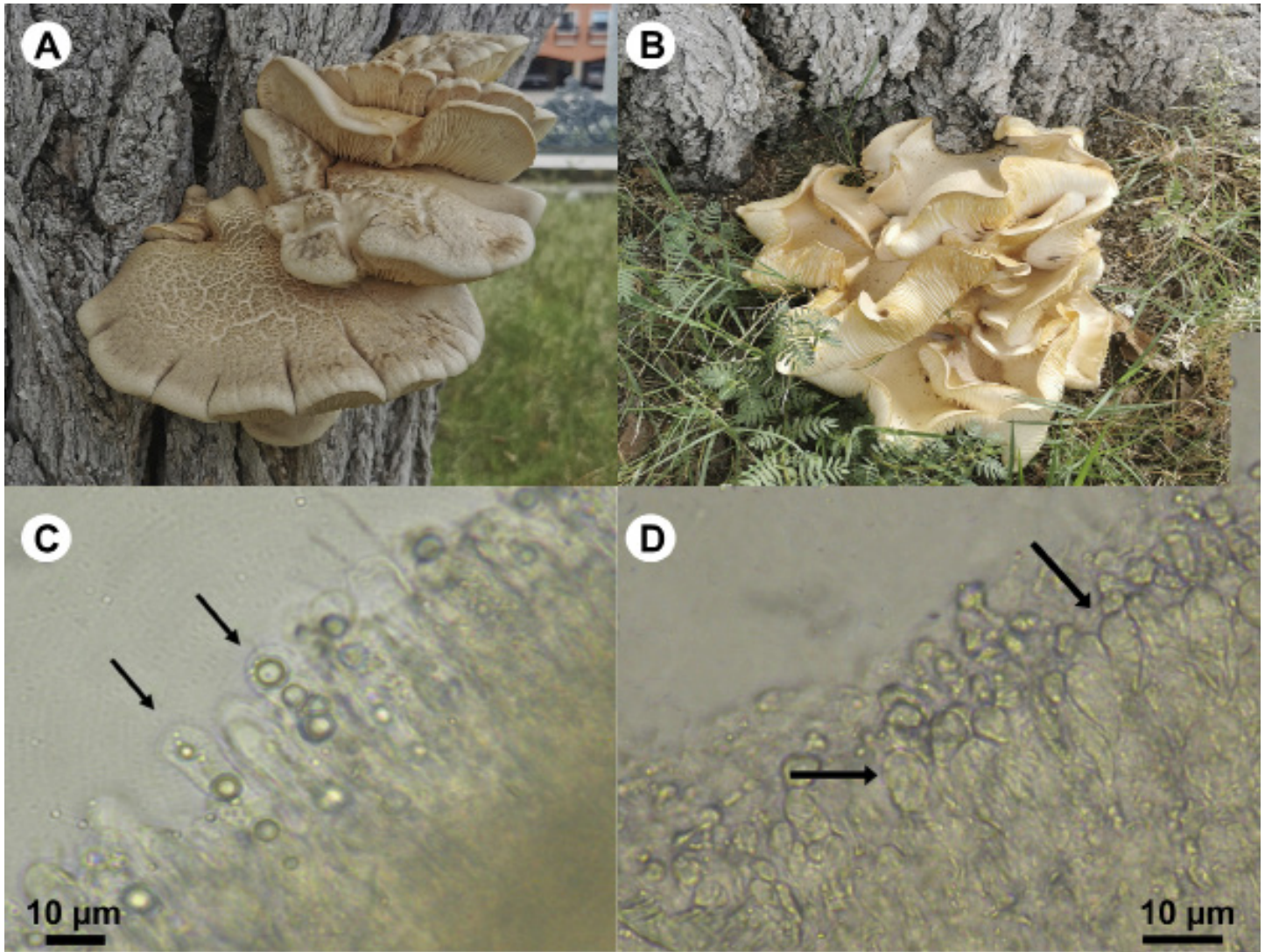


Figure 3: *Pleurotus cystidiosus* O.K. Miller, teleomorph. A. basidiome on an *Ulmus* sp. trunk; B. basidiome on the base of an *Ulmus* L. tree; C. pleurocystidia; D. cheilocystida.

tive, thin-walled, clamped hyphae 5-12 µm wide, slightly branched hyphae, occasionally with widened endings; cuticle of the pileus with abundant pileocystidia 28-40 × 8-10 µm, clavate to cylindrical, with a rounded apex, lamellae trama with clamped, hyaline hyphae 55-18 µm wide, mixed with amorphous, ochraceous elements.

Anamorph

Coremia developed abundantly on the surface of culture medium (PDA), gregarious, or occasionally in groups attached by the base, 0.8-2.5 mm in height, with a white stipe

and apex with a droplet of dark liquid; stipe formed by hyaline hyphae, septate and clamped, which fragment forming cylindrical arthrospores, brown or sometimes hyaline, 17-25 × 5-7.5 µm.

Habit: lignicolous, growing on an *Ulmus* sp. tree.

Distribution: Algeria (Stalpers et al., 1991), Argentina (Lechner et al., 2004), Brazil (Capelari, 1999), China (Zervakis et al., 2004), Cuba (Camino-Vilaró et al., 2018), Greece (Zervakis et al., 1992), India (Zervakis et al., 2004),



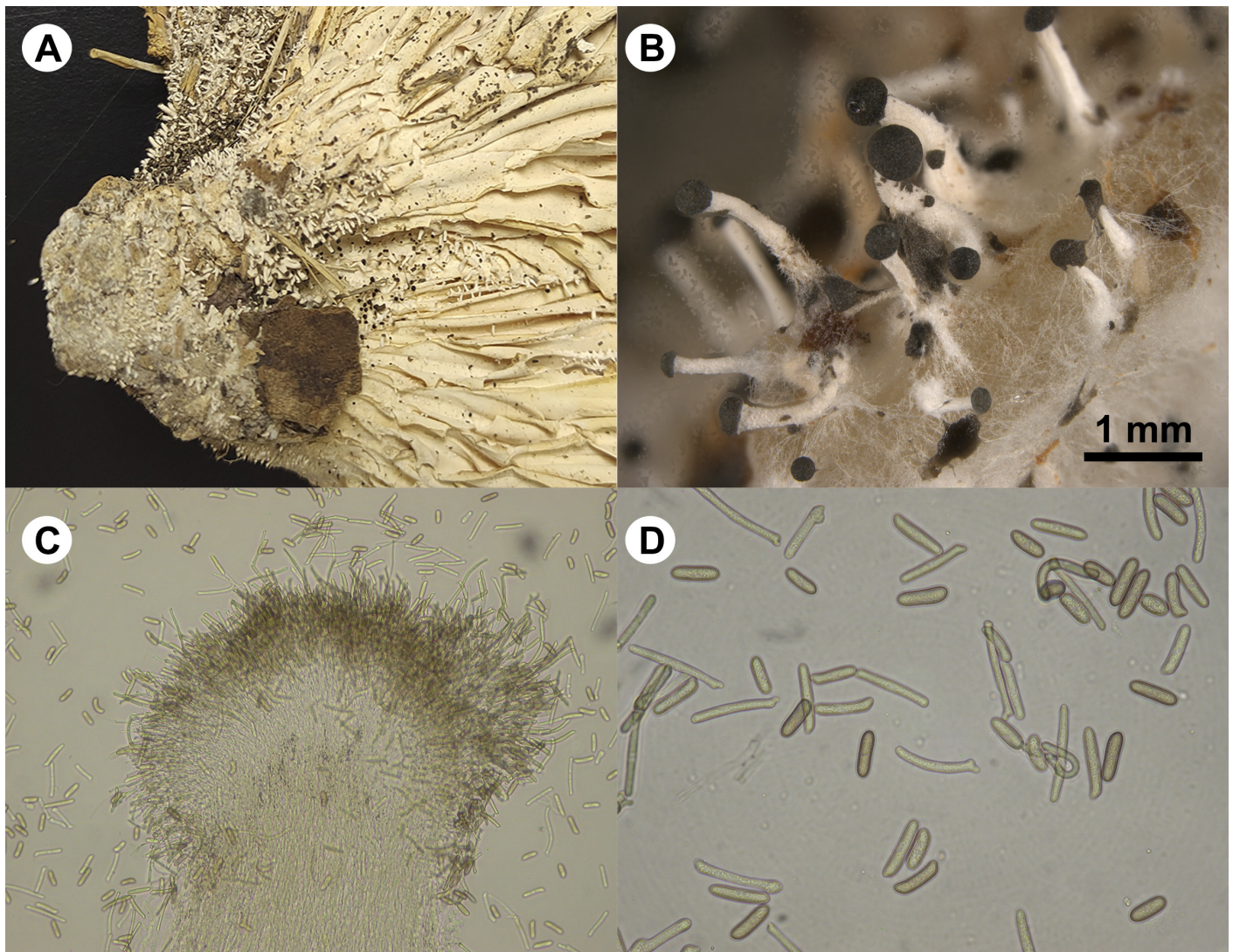


Figure 4: *Pleurotus cystidiosus* O.K. Miller, anamorph. A. coremia on basidiome; B. polyphocal microscopy of coremia on PDA; C. coremia in light microscopy; D. arthrospores in light microscopy.

Indonesia (Stalpers et al., 1991), Israel (Stajić et al., 2003), Japan (Zervakis et al., 2004), Pakistan (Hussain et al., 2015), Philippines, South Africa (Zervakis et al., 2004), Russia (Shnyreva and Shnyreva, 2015), Taiwan (Jong and Peng, 1975), Thailand (Zervakis et al., 2004), and United States of America (Miller, 1969).

Specimens examined: MEXICO. Chihuahua, municipality Ciudad Juárez, growing at the base of an *Ulmus* sp. tree, 6.IX.2021, *M. Lizárraga* s.n. (UACJ 3396), loc. cit., 25.VIII.2022, *M. Lizárraga* s.n. (UACJ 3397); loc. cit., *M. Lizárraga* s.n. (MEXU 30551). GenBank accession of the ITS sequence: OR532762.

Notes: *Pleurotus cystidiosus* can be easily confused with *P. smithii*, which also forms coremia. These two species can be differentiated by the presence of abundant pleurocystidia in *P. cystidiosus*, rarely only in the young stages of *P. smithii*, subglobose cheilocystidia in the teleomorph of *P. cystidiosus*, absent in *P. smithii*; as well as by the shorter segments $5-11(-15) \times 3.2-5(-6) \mu\text{m}$ of conidiophore elements in the anamorph of *P. smithii* (Guzmán et al., 1991). Other very similar species that also form coremia are *P. abalonus* which differs by its smaller basidiospores ($10.5-13.5 \times 3.8-5 \mu\text{m}$) and slender cheilocystidia ($7-8.5 \mu\text{m}$, diam.) (Guzmán, 2000). *Pleurotus purpureo-olivaceus* presents spherical and sessile conidiomata and

is restricted to Australia and New Zealand, as is *P. australis*, which is distinguished macroscopically by its dark reddish-brown pileus and microscopically by its smaller basidiospores (10.5-14 × 4-6 µm), pleurocystidia, and cheilocystidia (16-25 × 5 µm) (Segedin et al., 1995). *Pleurotus fuscosquamulosus* presents cylindrical to clavate cheilocystidia bearing an apical sterigma-like structure with an apical small capitulum (Torta et al., 2019), absent in our collections of *P. cystidiosus*.

Discussion

The species of *Pleurotus* included in the subgenus *Coremiopleurotus* are very similar and, therefore, difficult to distinguish using only morphological features. Accordingly, it is necessary to complement taxonomic studies with ITS sequences to delimit these species correctly. The close relationship between *P. cystidiosus* and *P. smithii* has been discussed from morphological and molecular perspectives. Capelari and Fungaro (2003) considered them synonyms based on Random Amplified Polymorphic DNA (RAPD). Zervakis et al. (2004) later established taxonomic independence using ITS sequences. Currently, the rDNA ITS region is considered the universal barcode for fungal species recognition (Schoch et al., 2012), and recent phylogenetic studies on the genus *Pleurotus* have shown the independence of both species and the close relationships within the subgenus, nowadays considered within clade one by Lin et al. (2022).

It is important to highlight the following observations of *Pleurotus cystidiosus* cited from Mexico: Sobal et al. (2007) listed a strain (CP-18) determined as *P. cystidiosus* from Veracruz, Mexico; however, the subsequent examination of the ITS sequence obtained from this sample confirmed its identity as *P. smithii* and not *P. cystidiosus* (Huerta et al., 2010). On the other hand, Camino-Vilaró et al. (2018) mentioned that *P. cystidiosus* is present in Mexico, referring to Guzmán et al. (1991). However, in this last article, these authors compared the material of *P. cystidiosus* from the USA with the Mexican material of *P. smithii*, the reason why in the present work, the occurrence of *P. cystidiosus* in Mexico is confirmed and supported by barcode sequences.

The species exhibits a broad geographical range, as it has been documented in numerous countries across the Americas, Eurasia, and Africa (Miller, 1969; Jong and Peng, 1975; Stalpers et al., 1991; Zervakis et al., 1992; 2004; Capelari, 1999; Stajić et al., 2003; Lechner et al., 2004; Hussain et al., 2015; Shnyreva and Shnyreva, 2015; Camino-Vilaró et al., 2018). Nevertheless, some reports lack barcoding sequences, such as the ones from Argentina (Lechner et al., 2004) and Brazil (Capelari, 1999). Barcode sequences are necessary to assess the global distribution of the species and validate the unsequenced *Pleurotus* species from Mexico. *Pleurotus cystidiosus* has been found on various hosts or substrates, including *Ficus carica* L., *Populus deltoides* W. Bartram ex Marshall, *Liquidambar styraciflua* L., *Quercus texana* Buckley, *Acer rubrum* L., *Zerkova serrata* (Thunb.) Makino, *Aphananthe aspera* (Thunb.) Planch., and *Koelreuteria henryi* Dümmer (Zervakis et al., 1992). Our material was collected from an *Ulmus* sp. tree. All this suggests that *P. cystidiosus* exhibits a wide host compatibility, indicating its ability to decompose various wood substrates.

From a biotechnological perspective, *P. cystidiosus* is a poorly studied species worldwide, unlike other species of the genus. Among the available studies, some carbon sources, different temperatures, and culture media have been evaluated, resulting in sucrose and 28 °C the best; and all the culture media evaluated showed no significant differences in mycelial growth (Hoa and Wang, 2015). In addition, various agroindustrial waste materials, including sugarcane bagasse (SB), corncob (CC), and sawdust from *Acacia* sp. wood, have been demonstrated to be effective for production of fruiting bodies; the most favorable outcomes were observed when using SB and CC (Hoa et al., 2015). Hoa et al. (2017) found that total phenolic contents and antioxidant activity depended on substrate formulas and drying methods. García et al. (2020) obtained a good amount of total phenolic contents and high antioxidant activity in *P. cystidiosus* extracts.

Despite the information shown above, there are no studies on the content of chitin, chitosan, or any other polysaccharide or metabolite with biological activity or biotechnological potential. For the previously mentioned



reasons, this is an opportunity to take advantage of our fungal resources in Mexico's arid and semiarid zones.

Conclusions

Pleurotus cystidiosus has a wide distribution and host range, and it can be mistaken for *P. smithii*. However, the presence of pleurocystidia in young states and long cylindrical cheilocystidia in the teleomorph of *P. cystidiosus*, separates it from *P. smithii* and other members of *Coremiopleurotus*. ITS barcode sequences are effective for the identification of these closely related species. The number of *Pleurotus* species known from Mexico rises to eight.

Author contributions

DLP and MLE collected and morphologically characterized the specimens. EHN performed all molecular procedures and analyses. All authors conceived and designed the study; contributed to the discussion, review, and approval of the final manuscript.

Founding

This research was funded by Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PA-PIIT)-Universidad Nacional Autónoma de México (UNAM), Project IA205323.

Acknowledgments

We thank the following members of Laboratorio de Secuenciación Genómica de la Biodiversidad of the Laboratorio Nacional de Biodiversidad (LANABIO) of the Instituto de Biología, Universidad Nacional Autónoma de México (IBUNAM): Lidia Cabrera for her technical support during tissue pulverization for DNA extraction, Susana Guzmán Gómez for her technical support in acquiring macroscopic photographs, and Laura Márquez and Nelly López Ortiz for their technical assistance in the sequencing process.

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