



Xerocomellus carmeniae (Boletales, Basidiomycota), a new fungus from northeastern Mexico

Xerocomellus carmeniae (Boletales, Basidiomycota), un nuevo hongo del noreste de México

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Abstract:

Background and Aims: *Xerocomellus* is a genus of the Boletaceae family, characterized by the small to medium, boletoid to gastroid basidiomata, usually with areolate pileus, and smooth to ornamented basidiospores. So far only two species are known from Mexico. The aim of this study is to describe a new species of *Xerocomellus*, based on morphological, molecular and ecological data.

Methods: Sampling of studied specimens was carried out in Nuevo León state, northeastern Mexico (2009 and 2016). Classic protocols for macrofungi were followed. Hand cut sections of specimens were made and mounted in KOH and Melzer reagent to observe microstructures. The identification of the putative host tree was made in the botanical herbarium CFNL of the Facultad de Ciencias Forestales, Universidad Autónoma de Nuevo León; the type of forest was identified according to field observations and satellite images. DNA was extracted from three different collections. The ITS region and the gene LSU were obtained and analyzed. The material was deposited in the mycological collections of the herbaria "José Castillo Tovar" (ITCV) of the Instituto Tecnológico de Ciudad Victoria and CFNL.

Key results: *Xerocomellus carmeniae* differs from other *Xerocomellus* species by the following combination of characteristics: boletoid basidiomata, reddish areolate pileus, yellowish stipe, and basidiospores of $10.5\text{--}13.6 \times 5.7\text{--}7.8 \mu\text{m}$, elongate, sometimes truncate.

Conclusions: *Xerocomellus carmeniae* is the third species of this genus known from Mexico and is putatively associated to *Quercus canbyi*. Some specimens show an aberrant form, but more studies are recommended to evaluate a possible transition to a secotoid form.

Key words: Agaricomycetes, boletes, fungi, new species, *Quercus*, symbiont fungi.

Resumen:

Antecedentes y Objetivos: *Xerocomellus* es un género de la familia Boletaceae, caracterizado por el basidioma pequeño a mediano, boletoide o gastroide, usualmente con píleo areolado y esporas lisas u ornamentadas. Hasta ahora se conocen dos especies de México. El objetivo de este estudio es describir una nueva especie de *Xerocomellus* basada en datos morfológicos, moleculares y ecológicos.

Métodos: El muestreo de los especímenes estudiados se realizó en el estado de Nuevo León, noreste de México (2009 y 2016). Se siguieron los protocolos clásicos para macro hongos. Se hicieron cortes manuales de especímenes y se montaron en KOH y reactivo de Melzer para observar microestructuras. La identificación del hospedero putativo se realizó en el herbario CFNL de la Facultad de Ciencias Forestales, Universidad Autónoma de Nuevo León; el tipo de bosque se identificó de acuerdo con observaciones de campo e imágenes de satélite. Se extrajo ADN de tres diferentes colecciones. Se obtuvieron y analizaron la región ITS y el gen LSU. El material se depositó en las colecciones micológicas de los herbarios "José Castillo Tovar" (ITCV) del Instituto Tecnológico de Ciudad Victoria y CFNL.

Resultados clave: *Xerocomellus carmeniae* se diferencia de otros *Xerocomellus* por la siguiente combinación de características: basidiomas boletoides, píleo areolados rojizo, estípite amarillentos y basidiosporas de $10.5\text{--}13.6 \times 5.7\text{--}7.8 \mu\text{m}$, elongadas, a veces truncadas.

Conclusiones: *Xerocomellus carmeniae* es la tercera especie de este género conocida de México y está putativamente asociada con *Quercus canbyi*. Algunos especímenes mostraron una forma aberrante, pero se recomiendan más estudios para evaluar una posible transición a una forma secotioide.

Palabras clave: Agaricomycetes, boletes, especie nueva, hongos, hongos simbiontes, *Quercus*.

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Introduction

Xerocomellus Šutara is a genus in the family Boletaceae characterized by boletoid, hypogeous to secotioid basidiomata, usually with small fruit body, sometimes cracked pileus, and smooth to ornamented spores; some species have hypogeous forms with ridged to globose-equinulate spores (Šutara, 2008; Smith et al., 2018; Frank et al., 2020). As other boletes, the species of *Xerocomellus* form ectomycorrhizas with members of Fagaceae and Pinaceae (Rinaldi et al., 2008; Tedersoo et al., 2010; Wu et al., 2016; Frank et al., 2020). The genus was segregated from *Xerocomus* Quél. by Šutara (2008), mainly by the basidiospore ornamentation and pileipellis arrangement. Recent phylogenetic studies place *Xerocomellus* close to *Hortiboletus* Simonini, Vizzini & Gelardi, in the clade 13 of the Boletoidae (Wu et al., 2016). According to recent research (Frank et al., 2020; Farid et al., 2021) and Index Fungorum (2022) information on the genus, 25 species are recognized worldwide.

Northern Mexico is known for its bolete diversity, with more than 150 species reported from the states of Tamaulipas, Nuevo León, and Coahuila (García-Jiménez and Garza-Ocañas, 2001; García-Jiménez, 2014). So far, no species of *Xerocomellus* have been described in Mexico using molecular approaches. Only two species are known from this country: *Xerocomellus chrysenteron* (Bull.) Šutara and *X. truncatus* (Singer, Snell & E.A. Dick) Klofac (Saldivar et al., 2021). Due to the diversity of hosts, forest ecosystems and elevations, more fungal species are expected to be found and which need to be described. During mycological exploration in the state of Nuevo León, some noteworthy boletes were found, characterized by the red areolate pileus and boletoid basidiomata. The aim of this study is to describe a new species of *Xerocomellus*, based on morphological, molecular and ecological data.

Material and Methods

Sampling

The mycological explorations were carried out in the state of Nuevo León, Mexico, during July-August 2009 and July-August 2016 (Fig. 1). The vegetation in the sampling zones is a mixed *Pinus-Quercus* forest. The identification of the putative host tree was made in the herbarium CFNL, of the

Facultad de Ciencias Forestales, Universidad Autónoma de Nuevo León, Mexico. The type of forest was identified according to field observations and satellite images. Methods for collecting, sampling and describing fungi followed Frank et al. (2020).

Hand cut sections were made from dried specimens and mounted in water, KOH 5% and Melzer's solution for microscopic description. The Methuen Handbook of Colour was used for color terminology (Kornerup and Wanscher, 1978). At least 30 microscopic structures (basidiospores, basidia and pileipellis) were measured with an optical microscope (Motic BA310, San Antonio, USA). The Q ratio, average length (L) and average width (W) were obtained for basidiospores according to Frank et al. (2020). The scanning electron microscope (JEOL JSM-6010PLUS, Tokyo, Japan) of El Colegio de la Frontera Sur (ECOSUR, Chetumal, Mexico) was used to observe basidiospores.

All the specimens are deposited in the mycological collections of the herbaria "José Castillo Tovar" (ITCV) of the Instituto Tecnológico de Ciudad Victoria and CFNL.

Amplification and sequencing

Total DNA was extracted from dried herbarium specimens using a modification of the Murray and Thompson (1980) protocol. The PCR amplification, based on Mullis and Faloona (1987), included 35 cycles with an annealing temperature of 54 °C, and was carried out with the ITS5 and ITS4 primers (White et al., 1990; Gardes and Bruns, 1993) for the ITS nrDNA region, and the LR0R and LR5 primers (Vilgalys and Hester, 1990; Cubeta et al., 1991) for the 28S nrDNA region (LSU).

The amplicons were verified by agarose gel electrophoresis. The gels were run for 1 h at 95 V cm⁻³ in 1.5% agarose and 1× TAE buffer (Tris Acetate-EDTA). The gel was stained with GelRed (Biotium, USA) and the bands were visualized in an Infinity 3000 transilluminator (Vilber Lourmat™, Marne-la-Vallée, France). The amplified products were purified with the ExoSAP Purification kit (Affymetrix Inc., Santa Clara, USA), following the manufacturer's instructions. They were quantified and prepared for the sequence reaction using a BigDye Terminator v. 3.1 (Applied Biosystems, Waltham, USA).



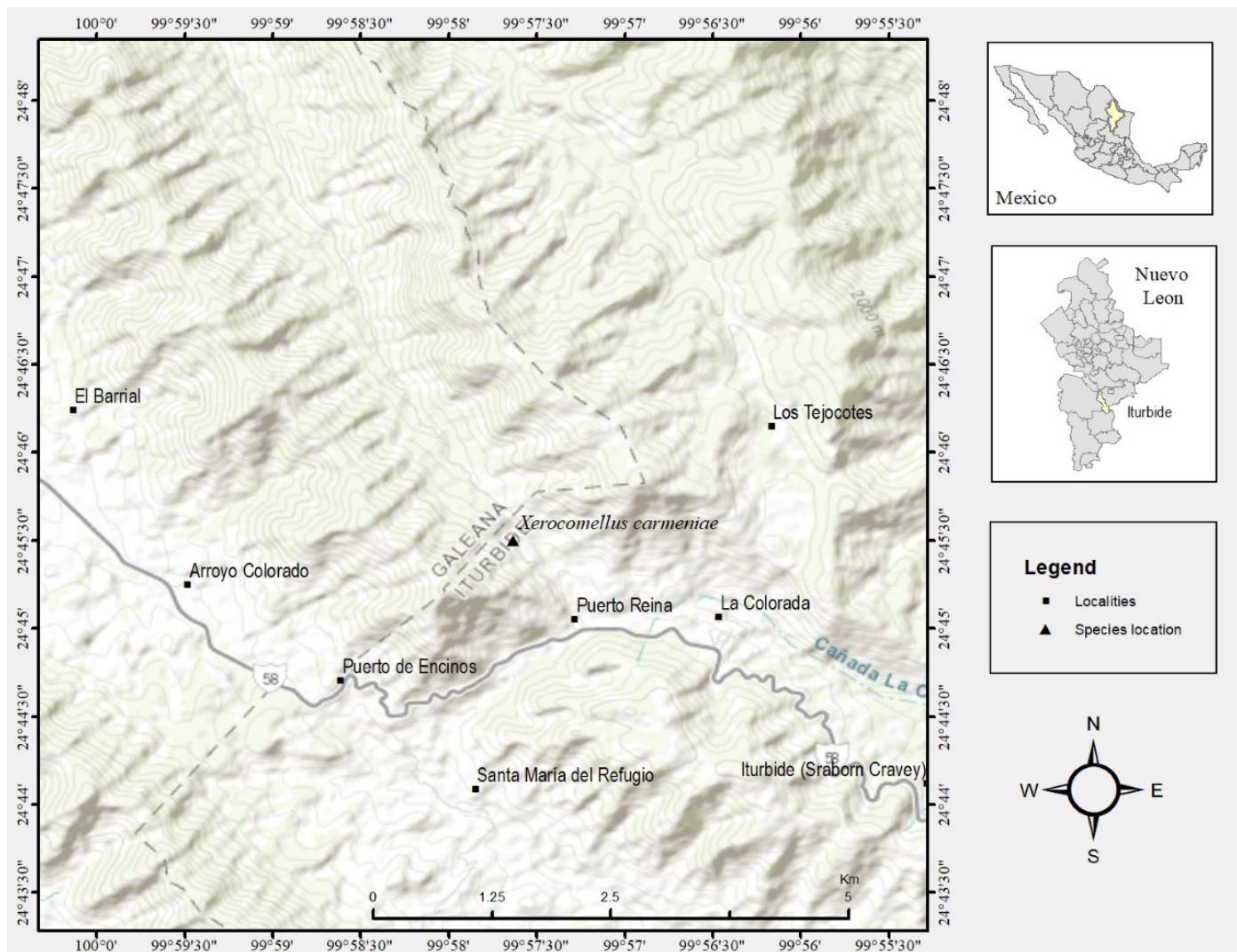


Figure 1: Distribution of *Xerocomellus carmeniae* Garza-Ocañas, J. García & de la Fuente.

These products were sequenced in both directions with an Applied Biosystems model 3730XL (Applied BioSystems, Waltham, USA), at the Instituto de Biología of the Universidad Nacional Autónoma de México (UNAM). The sequences obtained were compared with the original chromatograms to detect and correct possible reading errors.

Phylogenetic analyses

In order to study phylogenetic relationships, our newly produced sequences of three individuals of *Xerocomellus* were added to reference sequences of ITS and LSU (Table 1) deposited in the NCBI database (GenBank, 2022). Each gene region was independently aligned using the online

version of MAFFT v. 7 (Katoh et al., 2002, 2017; Katoh and Standley, 2013). Alignments were reviewed in PhyDE v. 10.0 (Müller et al., 2005), followed by minor manual adjustments to ensure character homology among taxa. The matrices were formed for ITS by 39 taxa (690 characters) and LSU by 23 taxa (592 characters). *Hortiboletus* was used as outgroup. The aligned matrices were concatenated into a single matrix (39 taxa, 1282 characters) with Mesquite v. 3.2 (Maddison and Maddison, 2021). Phylogenetic inferences were estimated with maximum likelihood in RAxML v. 8.2.10 (Stamatakis, 2014) with a GTR + G model of nucleotide substitution. To assess branch support, 1000 rapid bootstrap replicates were run with the GTRCAT model. For Bayesian posterior probability, the



Table 1: GenBank accession numbers corresponding to the sequences used in the phylogenetic analyses. In bold the accessions of the new species generated for this study.

Species name	Isolate/Voucher/strain	Locality	GenBank Accessions	
			ITS	nrLSU
<i>Xerocomellus aff. chrysenteron</i> (Bull.) Šutara	JLF5684	USA	MH168533	-----
<i>Xerocomellus amylosporus</i> (A.H. Sm.) J.L. Frank & N. Siegel	JLF3012	USA	KM213635	KU144742
<i>Xerocomellus atropurpureus</i> J.L. Frank, N. Siegel & C.F. Schwar	JLF3620	USA	KU144749	KU144750
<i>Xerocomellus atropurpureus</i> J.L. Frank, N. Siegel & C.F. Schwar	JLF4664	USA	KY659589	-----
<i>Xerocomellus atropurpureus</i> J.L. Frank, N. Siegel & C.F. Schwar	NS120712	USA	KM213641	KM213642
<i>Xerocomellus atropurpureus</i> J.L. Frank, N. Siegel & C.F. Schwar	JLF2795	USA	KM213638	KM213639
<i>Xerocomellus behrii</i> (Harkn.) Castellano, M.E. Sm. & J.L. Frank	OSC Trappel 17620	-----	KJ882290	-----
<i>Xerocomellus bolinii</i> J.A. Bolin, A.E. Bessette, A.R. Bessette, L.V. Kudzma, J.L. Frank & A. Farid	JAB 133	USA	MW675729	MW662582
<i>Xerocomellus bolinii</i> J.A. Bolin, A.E. Bessette, A.R. Bessette, L.V. Kudzma, J.L. Frank & A. Farid	JAB 95	USA	MW675735	MW662589
<i>Xerocomellus bolinii</i> J.A. Bolin, A.E. Bessette, A.R. Bessette, L.V. Kudzma, J.L. Frank & A. Farid	JAB 110	USA	MW675735	MW662580
<i>Xerocomellus carmeniae</i> Garza-Ocañas, J. García & de la Fuente	18219 Type ITCV	Mexico	ON392096	ON254917
<i>Xerocomellus carmeniae</i> Garza-Ocañas, J. García & de la Fuente	5193 ITCV	Mexico	ON392097	ON254918
<i>Xerocomellus carmeniae</i> Garza-Ocañas, J. García & de la Fuente	5192 ITCV	Mexico	ON392098	ON254919
<i>Xerocomellus cisalpinus</i> (Simonini, H. Ladurner & Peintner) Klofac	KR-M-0044831	Germany	MT006036	-----
<i>Xerocomellus cisalpinus</i> (Simonini, H. Ladurner & Peintner) Klofac	LUGO:ECC19102906	Spain	MW376718	-----
<i>Xerocomellus diffractus</i> (Simonini, H. Ladurner & Peintner) Klofac	JLF3554	USA	KU144769	KU144770
<i>Xerocomellus diffractus</i> (Simonini, H. Ladurner & Peintner) Klofac	JLF5745	USA	MH168534	-----
<i>Xerocomellus diffractus</i> (Simonini, H. Ladurner & Peintner) Klofac	NS120612	USA	KM213650	KM213651
<i>Xerocomellus dryophilus</i> (Thiers) N. Siegel, C.F. Schwarz & J.L. Frank	CFS3Nov11-2	USA	KM213645	KX534074
<i>Xerocomellus dryophilus</i> (Thiers) N. Siegel, C.F. Schwarz & J.L. Frank	JLF4134	USA	KX534076	KY659593
<i>Xerocomellus mcmurphyi</i> (Zeller & C.W. Dodge) Castellano, Saylor, M.E. Sm. & J.L. Frank	OSCMES 282	-----	KJ882289	KJ882292
<i>Xerocomellus mendocinensis</i> (Thiers) N. Siegel, C.F. Schwarz & J.L. Frank	JLF2275	USA	KM213653	KM213654
<i>Xerocomellus mendocinensis</i> (Thiers) N. Siegel, C.F. Schwarz & J.L. Frank	JLF3558	USA	KU144785	KU144786
<i>Xerocomellus mendocinensis</i> (Thiers) N. Siegel, C.F. Schwarz & J.L. Frank	CFS 1Nov11	USA	KM213656	KM213657
<i>Xerocomellus mendocinensis</i> (Thiers) N. Siegel, C.F. Schwarz & J.L. Frank	HDT 18392	USA	KM213655	-----
<i>Xerocomellus poederi</i> G. Moreno, Heykoop, Esteve-Rav., P. Alvarado & Traba	AH 44050 Type	-----	NR155971	-----
<i>Xerocomellus pruinatus</i> (Fr. & Hök) Šutara	G.M. 2015-09-23.4	Luxembourg	MW603181	MW603181
<i>Xerocomellus rainisie</i> (Bessette & O.K. Mill.) N. Siegel, C.F. Schwarz & J.L. Frank	OKM25915	USA	KM213664	-----



Table 1: Continuation.

Species name	Isolate/Voucher/strain	Locality	GenBank Accessions	
			ITS	nrLSU
<i>Xerocomellus ripariellus</i> (Redeuilh) Šutara	301	Spain	MN685108	-----
<i>Xerocomellus salicicola</i> C.F. Schwarz, N. Siegel & J.L. Frank	CS-5Mar2014-1	-----	KU144791	KU144792
<i>Xerocomellus salicicola</i> C.F. Schwarz, N. Siegel & J.L. Frank	UCSC1028	-----	KU144793	KU144794
<i>Xerocomellus sarnarii</i> Simonini, Vizzini & U. Eberh.	ML900101XE	Cyprus	MH011930	MH011930
<i>Xerocomus truncatus</i> (Singer, Snell & E.A. Dick) Klofac	HDT22426	USA	KU144796	-----
<i>Xerocomus truncatus</i> (Singer, Snell & E.A. Dick) Klofac	NY13857	USA	KU144795	-----
<i>Xerocomellus zelleri</i> (Murrill) Klofac	JLF2977	USA	KM213666	-----
<i>Xerocomellus zelleri</i> (Murrill) Klofac	Murrill Type	USA	KU144803	-----
<i>Hortiboletus campestris</i> (A.H. Sm. & Thiers) Biketova & Wasser	DD614	USA	MH168538	-----
<i>Hortiboletus cf. rubellus</i> (Krombh.) Simonini, Vizzini & Gelardi	JLF3093	USA	KU144805	-----

best evolutionary model for alignment was sought using PartitionFinder v. 2 (Lanfear et al., 2014, 2017; Frandsen et al., 2015).

Phylogenetic analyses were performed using MrBayes v. 3.2.6 x64 (Huelsenbeck and Ronquist, 2001). The information block for the matrix included two simultaneous runs, four Montecarlo chains, temperature set to 0.2 and sampling 10 million generations (standard deviation ≤ 0.1) with trees sampled every 1000 generations. The first 25% of samples were discarded as burn-in, and stationarity was checked in Tracer v. 1.6 (Rambaut et al., 2014). Trees were visualized and optimized in FigTree v. 1.4.4 (Rambaut, 2014). The phylogenetic tree is available at TreeBASE (accession number: TB2:S16457).

Results

Molecular analyses

The two simultaneous Bayesian runs continued until the convergence parameters were met, and the standard deviation fell below 0.001 after 4.5 million generations (Fig. 2). No significant changes in tree topology trace or cumulative split frequencies of selected nodes were observed after about 0.25 million generations, which were discarded as 25% burn-in. The analysis produced a phylogenetic tree where the new species is shown as a monophyletic clade with strong statistical support (1 Bayesian Posterior Probability (PP) and 100% bootstrap proportion (BP) for Maximum Likelihood).

Taxonomy

Basidiomycota

Agaricomycetes

Boletales

Boletaceae

Xerocomellus carmeniae Garza-Ocañas, J. García & de la Fuente sp. nov. Figs. 3, 4.

TYPE: MEXICO. Nuevo León, municipality Iturbide, Puerto a Pastores, *Pinus-Quercus* forest, under *Q. canbyi*, 16.VII.2016, J. García 18219 (holotype: ITCV!, isotype: CFNL!). Mycobank: MB843147. Genbank: ITS: ON392096; LSU: ON254917.

Xerocomellus carmeniae is characterized by the following combination of features: boletoid basidioma, reddish areolate pileus, context yellowish, staining blue when cut, truncate, elongate to ellipsoid basidiospores, 10.5-13.6 \times 5.7-7.8 μm .

Basidiomata boletoid, sometimes with a poorly developed stipe; pileus 18-32 mm diameter, vivid red to deep red (11A8-11C8), light brown to brown (6D4-6E4)



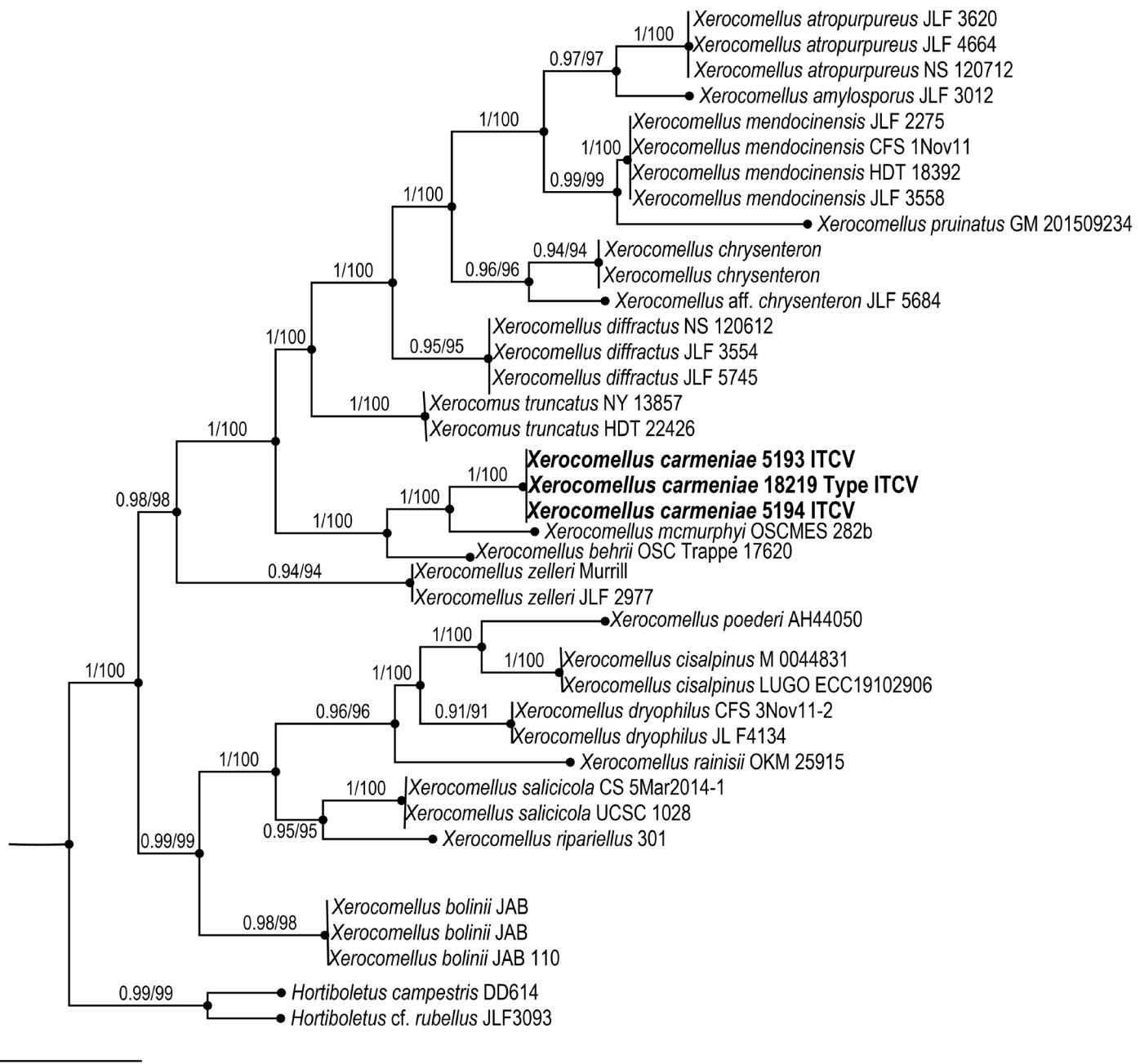


Figure 2: Bayesian inference phylogram of ITS-LSU sequences data. Posterior probability (left of slash) from Bayesian analysis and Bootstrap support (right of slash).

background pale yellow (4A6), slightly convex to flattened, margin irregular; context yellowish (4A8), bruising greyish green (28B5) to greyish blue (24D5) when young, unchanging when mature; tubes irregularly arranged, emarginated sometimes, yellowish (30A8) to greyish green (27E5), with angular to irregular pores, sometimes covered by a thin whitish layer near the stipe apex; stipe 13-34 × 0.5-10 mm, irregular and sinuous, pale brown

(5D4) to pale yellow (4A7), with small reddish (11A8) scales, sometimes becoming pale brown (5D4) to grey brown (5D3) at the slender base; pileipellis 105-174 µm thick, composed of compacted interwoven chains of cylindrical to subglobose hyphae, 10-22 µm diameter, with erect and clavate to fusoid terminal cells, brownish to yellowish in KOH 5% and Melzer's reagent, with granular intracellular content; hymenophoral trama slightly

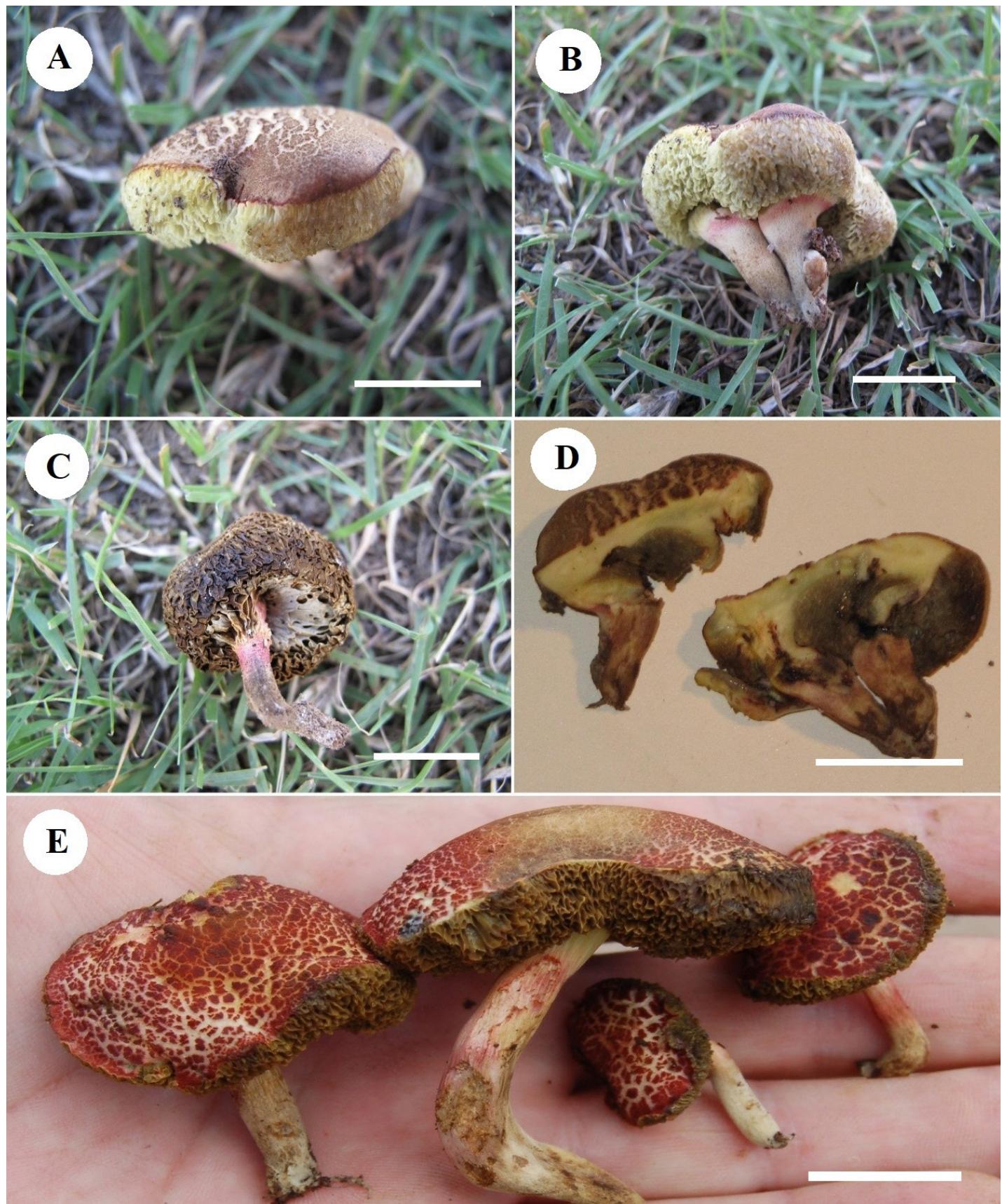


Figure 3: *Xerocomellus carmeniae* Garza-Ocañas, J. García & de la Fuente (holotype). A-B. details of the basidiomata; C. details of the tubes; D. context; E. basidiomata and details of the pileus. Scale: 10 mm.

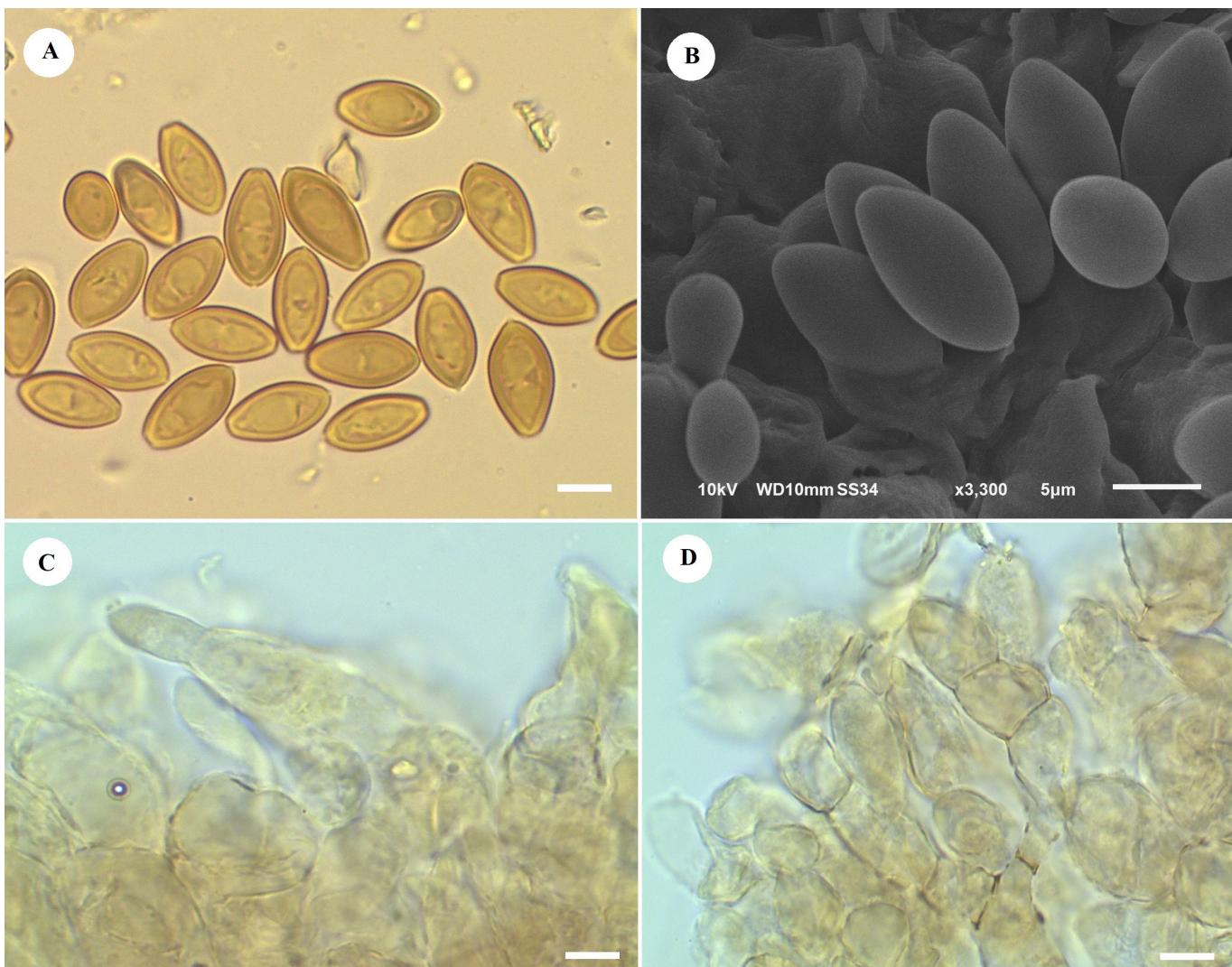


Figure 4: *Xerocomellus carmeniae* Garza-Ocañas, J. García & de la Fuente (holotype) A-B. basidiospores; C-D. elements of pileipellis. Scale=A:10 μ m; B: 5 μ m; C-D: 10 μ m.

divergent, with cylindric and loosely interwoven hyphae, 2-6 μ m in diameter, hyaline, thin-walled; basidia clavate to subclavate, 25-36 \times 11-18 μ m, hyaline, 4-spored, with sterigma projecting up to 3 μ m long, thin-walled; hymenial cystidia not observed; basidiospores 10.5-13.6 \times 5.7-7.8 μ m (L=11.05, W=6.90, Q=1.60, N=30), truncate, elongate, brown in KOH and Meltzer, guttulate, sometimes truncate, smooth and thick-walled.

Habit and habitat: scattered in small groups, mixed *Pinus*-*Quercus* forest under *Quercus canbyi* Trel.

Distribution: only known from Nuevo León, Mexico.

Etymology: named *carmeniae* in honor of María del Carmen A. Medina Cortés, who found the specimens of this study.

Additional material examined: MEXICO. Nuevo León, municipality Iturbide, Puerto Pastores, 02.VIII.2009, F. Garza-Ocañas 5192 (CFNL), 5193 (CFNL).

Notes: the new species differs from other members of *Xerocomellus* such as *Xerocomellus macmurphyi* (Zeller & C.W. Dodge) Castellano, Saylor, M.E. Sm. & J.L. Frank and *Xerocomellus behrii* (Harkn.) Castellano, M.E. Sm. & J.L. Frank, due to the globose and hypogeous basidiomata



with spinose basidiospores (Smith et al., 2018; Frank et al., 2020). It also differs from the boletoid to secotioid basidiomata of *X. amylosporus* (A.H. Sm.) J.L. Frank & N. Siegel, because it has amyloid and larger basidiospores (Frank et al., 2020).

Discussion

Boletaceae is one of the most common groups of fungi present in temperate forest (Ayala-Vásquez et al., 2022). Despite the usually developed boletoid, lamellate, epigeous fruit body, several species grow underground as false truffles or secotioid species (Thiers and Trappe, 1969; Binder and Hibbett, 2006; Smith et al., 2018). Most of them form ectomycorrhizal associations with angiosperms and gymnosperms (Hasselquist et al., 2011; Wu et al., 2016). The boletes are one of the major groups of fungi present in northeastern Mexican temperate forest with about 150 described species (García-Jiménez, 2014). More species are being described from the Mexican temperate zone, indicating the great species richness in Mexico (Ayala-Vásquez et al., 2018).

The genus *Xerocomellus* shows typically epigeous boletoid taxa; nevertheless, few hypogeous and secotioid species are known (Smith et al., 2018; Frank et al., 2020). The most remarkable features of the new species are the boletoid basidiomata, the reddish pileus, and the truncate basidiospores. Interestingly, *Xerocomellus carmeniae* is phylogenetically closer to hypogeous species such as *Xerocomellus macmurphyi* and *Xerocomellus behrii*, than to secotioid species such as *Xerocomellus amylosporus* or with truncate spores such as *Xerocomellus mendocinensis* (Thiers) N. Siegel, C.F. Schwarz & J.L. Frank (Frank et al., 2020). Despite *Xerocomellus carmeniae* has a boletoid basidiomata, some collections show an aberrant form that could represent a transition to a secotioid form (Fig. 5).

Even when the hymenium is not completely enclosed within a persistent peridium, the absence of hymenial cystidia and the basidiospores without hilar depression could indicate a possible loss of the capability for active dispersal. These characteristics are found in the truly secotioid boletes (Thiers and Trappe, 1969; Thiers, 1984). Other *Xerocomellus* species such as *X. amylosporus* and *X. atropurpureus* J. L. Frank, N. Siegel & C.F. Schwarz



Figure 5: *Xerocomellus carmeniae* Garza-Ocañas, J. García & de la Fuente (F. Garza-Ocañas 5192) showing an aberrant basidioma. Scale=10 mm.

also have a gastroid morphological variation (Frank et al., 2020). More collections are needed to clarify the possible secotioid transition of this new species.

The sequestrate Boletales are still poorly studied when compared with their epigeous relatives. In Mexico, only some species of *Octaviania* Vittad., *Melanogaster* Corda and *Rhizopogon* Fr. (Trappe and Guzmán, 1971; Cázares et al., 1992; 2008) have been described. Considering Mexico as a diversification center of the genera *Quercus* L. and *Pinus* L, trees which form association with several species of mycorrhizal fungi (Trappe et al., 2009), more studies about the Mexican symbiotic fungi are recommended.

Author contributions

FGO and JGJ collected the material. JIF, CRMG, and OAV wrote the manuscript. CRMG made the molecular analyses. GGG, JIF, JGJ and OAV described the species. All authors contributed to the manuscript.

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