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The genus *Xerocomellus* (Boletales, Boletaceae) in Mexico: a new species, a new record, and notes on its biocultural importance

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Abstract

Mexico has a great diversity of Boletaceae species. However, some genera, including those of the *Xerocomellus*, have been overlooked. This work presents a new species and a new record of *Xerocomellus*, together with a key to the *Xerocomellus* in Mexico. The new species of *Xerocomellus* is proposed based on morphological and phylogenetic analyses of DNA sequences from four genes (ITS, LSU, *rpb1, and tef1*). *Xerocomellus piedracanteadensis sp. nov.* forms an independent lineage from other species of the genus and is characterized by medium-sized basidiomata, dry, tomentose to rivulose or areolate pileus surface with whitish flesh staining pale yellow to pale red, with basidiospores ranging from (8)10–14(15) × (3)–5 µm, pileipellis formed by a trichoderm 300 µm thick, with terminals cells 13–55 × (8)10–23 µm, cylindrical, ovoid, subglobose to mammillate with rounded or acute apex, coarsely encrusting in lower elements ringed look wall, dark brown. Additionally, the geographical range of *Xerocomellus bolinii,* previously known only from Florida, the USA*,* is broadened to Central Mexico, and its edibility is reported for the first time. Photographs and a detailed description of the new species, the new record, and their phylogenetic analysis results are presented. An updated key for the *Xerocomellus* in Mexico is also presented.

Key words: coniferous mixed forests, ectomycorrhizal fungi, edible wild mushrooms, one new species, pine-oak forest

Introduction

The family Boletaceae arose approximately 138 Mya, holding one of the largest diversity of mushrooms worldwide (Tremble *et al.* 2023). Currently there are eight subfamilies: Austroboletoideae G. Wu & Zhu L. Yang (2014:69), Boletoideae G. Wu & Zhu L. Yang (2014:69), Chalciporoideae G. Wu & Zhu L. Yang (2014:69), Leccinoideae G. Wu & Zhu L. Yang (2014:69), Pseudoboletoideae G. Wu, Halling & Zhu L. Yang (2023-10), Suillelloideae G. Wu, Halling & Zhu L. Yang (2023-10), Xerocomoideae Singer (2014:69), and Zangioideae G. Wu & Zhu L. Yang (2014:69), (Wu *et al.* 2016, Wu *et al.* 2023). The genus *Xerocomellus* Sutara (2008:60), which belongs to the Boletoideae subfamily, has *Hortiboletus* Simonini, Vizzini & Gelardi as a sister genus (Wu *et al.* 2016). *Xerocomellus* was proposed by Šutara (2008) with *X. chrysenteron* (Bull.) Šutara as the type species. Šutara (2008) separated *Xerocomus* Quél. (1887:477) from *Xerocomellus*. This last genus distinguished by small to medium pileus, surface pileus at first smooth, subtomentose, velvety, hymenium adnate to sinuate, yellow, yellow-olivaceous to brownish yellow, staining blue immediately when bruised; basidiospores smooth rarely longitudinal ridges, subfusoid or fusoid and sometimes truncate never bacilliform, pileipellis is arranged as a palisoderm of vertically arranged, incrusted hyphae; (Wu *et*

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al. 2016). Currently, *Xerocomellus* has 29 recognized species, 13 of which have been reported from North America (Frank *et al.* 2020), 10 from Europe (Ariyawansa *et al.* 2015, Šutara 2008), and seven species from Asia (Das *et al.* 2023, Wu *et al.* 2016, Xue *et al.* 2024). The species belonging to this genus have paramount ecological importance in the functioning of the forest ecosystem because they form an ectomycorrhizal mutualistic symbiosis, mainly in trees belonging to Fagaceae. Their ectomycorrhizal roots are monopodial-pyramidal or coralloid, with rhizomorphs, and frequently ramified, white, brown, yellowish brown to green colour with mantle-type with a ring-like arrangement of hyphal bundles (Brand 1989). Some *Xerocomellus* species have anthropocentric importance as a food source around the world. Currently, nine edible *Xerocomellus* species have been reported (Li *et al.* 2021, Martinez-Reyes *et al.* 2023). Additionally, medicinal properties have been found in species belonging to this genus, such as anticancer activity, antimicrobial properties, mainly against colon cancer, and antioxidant activity (Ozgur *et al.* 2021, Tian *et al.* 2022). In Mexico, the species of this genus have biocultural importance in many original cultures; however, in general, they have been overlooked. Pérez-Moreno *et al.* (2010) reported several edible ethnotaxa belonging to *Xerocomellus* from Central Mexico. *Xerocomellus truncatus* (Singer, Snell & E.A. Dick) Klofac has been reported from the Trans-Mexican Volcanic Belt and the Sierra Madre del Sur, associated mainly with *Abies* (Ayala-Vásquez 2021, Saldivar *et al.* 2021). *Xerocomellus dryophilus* (Thiers) N. Siegel, C.F. Schwarz & J.L. Frank is known to be distributed in Baja California in the northwest part of the country associated with *Quercus agrifolia* Neé (Ayala-Sánchez *et al.* 2015). *Xerocomellus carmeniae* Garza-Ocañas, J. García & de la Fuente, was described by Garza-Ocañas *et al.* (2022) from northeastern Mexico. *Xerocomellus perezmorenoi* Ayala-Vásquez, Martinez-Reyes, was recently described by Martinez-Reyes *et al.* (2023) from Central Mexico found in *Pinus-Quercus* forests.

In recent explorations in mixed coniferous forests and *Pinus-Quercus* forests in central Mexico, *X. piedracanteadensis* was collected as a new species with biocultural importance as a food resource by local people in its type locality. Also, *X. bolinii* was first reported in Mexico, and the distribution range was extended. This latter species was only known in Florida, the USA, at an altitude of 40 m (Farid *et al.* 2021); in Mexico, it is distributed at 2785 to 3200 m. Molecular studies are presented with four genes supporting the new species and the new record for Mexico. Additionally, we present an updated key for *Xerocomellus* in Mexico.

Material & methods

Fieldwork and morphological analyses

After recording important information (Rathnayaka *et al.* 2024), basidiomata were collected in the Piedra Canteada Region, in the Tlaxcala state of central Mexico. In addition, some parasitized specimens of the studied species were also collected. The vegetation types of the study area are mixed coniferous and *Pinus-Quercus* forests. They are dominated by *Abies religiosa* Kunth Schltdl. et Cham., *Pinus teocote* Schiede ex Schltdl., *P. montezumae* Lamb, *P. pseudostrobus* Lindl., *Q. laurina* Humb et Bonpl., and *Q. aff. crassipes*. Protocols for sampling macrofungi were according to Lodge *et al.* (2004). After, the samples are dried at a temperature of 45°C (Hu *et al.* 2022). The colour descriptions were based on Kornerup and Wanscher (1978). Sections were cut from dried specimens, and temporary preparations were made using 5% KOH, Congo red, and Melzer's reagent. The size of the basidia, cystidia, and basidiospores, were determined by measuring at least 50 elements each. The abbreviation Q means the length/width ratio of a basidiospore in the side view. The material studied is deposited in the mycological herbaria MEXU at the National Autonomous University of Mexico in Mexico City.

DNA extraction, PCR amplification, and sequencing

Five specimens of *X. piedracanteadensis* and two specimens of *X. bolini* were studied for genetic analysis. Genomic DNA was obtained with the CTAB method (Martínez-González *et al.* 2017) using 2–3 mg of dried tissue. DNA quantification was performed with Nanodrop 2000c (Thermo, the USA). We prepared dilutions from each sample at 20 ng/µL to amplify the next for 4 regions: Internal Transcribed Spacer (nrITS), nuclear large subunit ribosomal DNA (LSU), the largest subunit of RNA polymerase II gene (*rpb1*), and translation elongation factor 1-α gene (*tef*1). The PCR reaction contained the following: enzyme buffer 1 x, Taq DNA polymerase, 0.8 mM deoxynucleoside triphosphates (0.2 mM each), 100 ng DNA, 20 pmol of each primer, and 2 units of GoTaq DNA (Promega, the USA), with a final volume of 15 µL. The PCR products were verified by agarose gel electrophoresis run for 1 h at 95 V cm-3 in 1.5%

agarose and 1 x TAE buffer (Tris Acetate-EDTA). The products were then dyed with GelRed (Biotium, the USA) and viewed in a transilluminator (Infinity 3000 Vilber, Loumat, Germany). Finally, the products were purified using the ExoSap Kit (Affymetrix, the USA) according to the manufacturer's instructions and were prepared for the sequencing reaction using the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied BioSystems). Sanger sequencing was carried out by Macrogen Inc. (Seoul, Korea). The sequences were analyzed, edited, and assembled using BioEdit v. 1.0.5 (Hall 1999) to create consensus sequences. The consensus sequences were compared with those in the GenBank database of the National Center for Biotechnology Information (NCBI) using the BLASTN 2.2.19 tool (Zhang *et al.* 2000). The same procedure was followed for the parasitized specimens to identify the parasite with molecular analysis.

Phylogenetic analyses

In the phylogenetic analysis, our newly produced sequences of six individuals of *Xerocomellus* were added to reference sequences of ITS, LSU, *rpb1*, and *tef1* (Table 1) deposited in the NCBI database (http://www.ncbi.nlm.nih.gov/genbank/). Each gene region was independently aligned using the online version of MAFFT v7 (Katoh *et al.* 2002; 2017; Katoh & Standley 2013). Alignments were reviewed in PhyDE V. 10.0 (Müller *et al.* 2005), followed by minor manual adjustments to ensure character homology between taxa. The matrices were formed for 53 ITS sequences (605 characters), 51 LSU sequences (610 characters), 22 *rpb1* sequences (684 characters), and 22 *tef1* sequences (600 characters). *Hortiboletus* sp. was used as the outgroup. The aligned matrices were concatenated into a single matrix (57 taxa, 2499 characters) with Mesquite v. 3.70 (Maddison & Maddison 2021). Eight partitioning schemes were established: one for the ITS, one for the LSU, three to represent the codon positions of the gene region *rpb1*, and three for *tef1* gene region, which were established using the option to minimize the stop codon with Mesquite v. 3.70 (Maddison & Maddison 2021). Phylogenetic inferences were estimated with maximum likelihood in Rax-ML 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 GTRGAMMA model. For Bayesian posterior probability, the best evolutionary model for alignment was sought using Partition Finder v.2 (Lanfear *et al.* 2014; 2017; Frandsen *et al.* 2015). Phylogenetic analyses were performed using MrBayes v. 3.2.6 x64 (Huelsenbeck & 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 *et al.* 2014).

TABLE 1. Species name, voucher, geographic location, and GenBank accession numbers of ITS, LSU, *rpb*1, and *tef*1 sequences used in the molecular analysis. NA refers to the unavailability.

Taxon	Voucher	Country	ITS	LSU	*RPB1*	*TEF1*	References
Xerocomellus amylosporus	JLF3012	The USA	KM213635	KU144742	NA	NA	Frank *et al.* 2020
X. amylosporus	iNAT:18617460	The USA	OL602056	OL602056	NA	NA	Unpublished
X. amylosporus	UBC:F28014	The USA	MZ817039	MZ817039	NA	NA	Unpublished
X. amylosporus	SAT-18-273-02	NA	MT946693	MT946693	NA	NA	Unpublished
X. atropurpureus	NS120712	The USA	KM213641	KM213642	NA	NA	Frank *et al.* 2020
X. atropurpureus	JLF3620	The USA	KU144749	KU144750	MW737517	MW737495	Frank *et al.* 2020
X. atropurpureus	NY1193858	NA	NA	KF030271	KF030366	KF030416	Nuhn *et al.* 2013
X. bolinii	**MEXU 30421**	**México**	**OL763321**	**OL763327**	**QS15261**	**QS15262**	**In this study**
X. bolinii	**MEXU 30422**	**México**	**QS15259**	**QS15260**	**QS15264**	**QS15265**	**In this study**
X. bolinii	JAB_133	The USA	MW675729	MW675729	NA	NA	Farid *et al.* 2021

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TABLE 1. (Continued)

Taxon	Voucher	Country	ITS	LSU	*RPB1*	*TEF1*	References
X. bolinii	JAB_110	The USA	MW675728	MW675728	MW737507	NA	Farid *et al.* 2021
X. bolinii	JAB_95	The USA	MW675735	MW675735	MW737511	MW737491	Farid *et al.* 2021
X. brunneus	MHKMU L.P. Tang 3774 holotype	China	PP189878	PP179422	PP195246	PP230532	Xue *et al.* 2024
X. brunneus	NA	China	NA	KF112340	KF112524	KF112170	Xue *et al.* 2024
X. cisalpinus	LUGO:ECC19102906	Spain	MW376718	MW376718	NA	NA	Unpublished
X. cisalpinus	KR-M-0044831	Germany	MT006036	MT006036	NA	NA	Unpublished
X. cisalpinus	AT2005034	Finland	NA	KF030354	KF030367	KF030417	Nuhn *et al.* 2013
X. chrysenteron	DQ533981	The USA	DQ533981	NA	NA	NA	Frank *et al.* 2020
X. chrysenteron	18177	Italy	JF908799	NA	NA	NA	Frank *et al.* 2020
X. cf. chrysenteron	JLF5684	The USA	MH168533	NA	NA	NA	Frank *et al.* 2020
X. diffractus	NS120612	The USA	KM213650	KM213651	NA	NA	Frank *et al.* 2020
X. diffractus	JLF3554	The USA	KU144769	KU144770		NA	Frank *et al.* 2020
X. diffractus	JLF5745	The USA	MH168534	NA	MW737519	NA	Frank *et al.* 2020
X. dryophilus	CFS3Nov11-2	The USA	KM213645	KX534074	NA	NA	Frank *et al.* 2020
X. himalayanus	DC 21-56	India	OQ847959	OQ847979	NA	NA	Das *et al.* 2023
X. himalayanus	DC 21-12	India	OQ847832	OQ847962	NA	NA	Das *et al.* 2023
X. dryophilus	JLF4134	USA	KX534076	KY659593	NA	MW737493	Frank *et al.* 2020
X. mendocinensis	JLF2775	The USA	KM213653	KM213654	NA	NA	Frank *et al.* 2020
X. mendocinensis	CFS1Nov11_1	The USA	KM213656	KM213657	NA	NA	Frank *et al.* 2020
X. mendocinensis	CFS10Nov2012_1	The USA	KM213659	KM213660	NA	NA	Frank *et al.* 2020
X. mendocinensis	JLF3558	The USA	KU144785	KU144786	NA	NA	Frank *et al.* 2020
X. mendocinensis	HDT18392	The USA	KM213655		NA	NA	Frank *et al.* 2020
X. pruinatus	G.M. 2015-09-23	Germany	MW603181	MW603181	NA	NA	Unpublished
X. ripariellus	301	Spain	MN685108	MN685108	NA	NA	Unpublished
X. ripariellus	VDKO0404	Belgium	NA	NA	NA	MH614746	Unpublished
X. rainisiae	OKM25915	The USA	KM213664	NA	NA	NA	Frank *et al.* 2020
X. rainisiae	JLF3523	The USA	KU144789	KU144790	MW737515	NA	Frank *et al.* 2020
X. roseonigrescens	GDGM43238, hopotype	China	NA	NG_059586	KT220591	KT220595	Xue *et al.* 2024
X. roseonigrescens	ZT13553	China	NA	KT220589	KT220592	KT220596	Xue *et al.* 2024
X. salicicola	CS_5Mar2014-1	The USA	KU144791	KU144792	NA	NA	Frank *et al.* 2020
X. salicicola	UCSC-F-1028	The USA	KU144793	KU144794	NA	NA	Frank *et al.* 2020
X. salicicola	B391	The USA	MW675727	MW662569	MW737496	NA	Farid *et al.* 2021

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TABLE 1. (Continued)

Taxon	Voucher	Country	ITS	LSU	***RPB1***	***TEF1***	References
X. sarnarii	ML900101XE	Italy	MH011930	MH011930	NA	NA	Loizides *et al.* 2019
X. tenuis	MHKMU R. Xue 100, holotype	China	PP189876	PP179418	PP195245	PP230529	Xue *et al.* 2024
X. tenuis	MHKMU R. Xue 94	China	PP189877	PP179416	PP195242	PP230530	Xue *et al.* 2024
X. truncatus	Halling6878	The USA	KM213665	NA	NA	NA	Frank *et al.* 2020
X. truncatus	HDT22426	The USA	KU144798	NA	NA	NA	Frank *et al.* 2020
X. truncatus	SGT-2012	The USA	JX030249	JX030249	NA	NA	Frank *et al.* 2020
X. zelleri	JLF2977	The USA	KM213666	KU144799	NA	NA	Frank *et al.* 2020
X. zelleri	W.A. 105	The USA	KU144803		NA	NA	Frank *et al.* 2020
X. perezmorenoi	MEXU 30410	Mexico	OK350679	OK350681	OQ015753	OQ017656	Martínez-Reyes *et al.* 2023
X. perezmorenoi	MEXU 30411	Mexico	OK350680	OK350682	OQ015754	OQ017657	Martínez-Reyes *et al.* 2023
X. perezmorenoi	MEXU 30412	Mexico	OQ077193	OQ101206	OQ015755	OQ017658	Martínez-Reyes *et al.* 2023
X. piedracanteadensis	**MEXU-HO 30418**	**Mexico**	**OL763323**	**OL763329**	**QS15249**	**QS15250**	**In this study**
X. piedracanteadensis	**MEXU-HO 30417, holotype**	**Mexico**	**OL763322**	**OL763328**	**QS15246**	**QS15247**	**In this study**
X. piedracanteadensis	**MEXU-HO 30419**	**Mexico**	**OL763324**	**OL763330**	**QS15252**	**QS15253**	**In this study**
X. piedracanteadensis	**MEXU-HO 30420**	**Mexico**	**OL763325**	**OL763331**	**QS15255**	**QS15256**	**In this study**
X. piedracanteadensis	**MEXU-HO 30430**	**Mexico**	**OL763326**	**OL763332**	**QS15258**	**QS15259**	**In this study**
X. poederi	AH 44050	Spain	NR_155971	NG_060000	NA	NA	Unpublished
Hortiboletus cf. rubellus	JLF3093	The USA	KU144805	NA	NA	NA	Frank *et al.* 2020
H. campestris	DD614	The USA	MH168538	MH203598	NA	NA	Frank *et al.* 2020
H. campestris	MICH KUO-08240502	The USA	NA	MK601740	NA	MK721094	Kuo & Ortiz-Santana 2020

Note: The sequences generated in this research are in bold.

Phylogenetic analyses

For the phylogenetic analysis, our newly produced sequences of six individuals of *Xerocomellus* were added to reference sequences of ITS, LSU, *rpb*1, and *tef*1 (Table 1) deposited in the NCBI database (http://www.ncbi.nlm.nih.gov/genbank/). Each gene region was independently aligned using the online version of MAFFT v7 (Katoh *et al.* 2002, 2017, Katoh & Standley 2013). Alignments were reviewed in PhyDE V. 10.0 (Müller *et al.* 2005), followed by minor manual adjustments to ensure character homology between taxa. The matrices were formed with 53 ITS sequences (605 characters), 51 LSU sequences (610 characters), 24 *rpb*1 sequences (684 characters), and 23 *tef*1 sequences (600 characters). *Hortiboletus* sp. was used as the outgroup. The aligned matrices were concatenated into a single matrix (51 taxa, 2,499 characters) with Mesquite v. 3.70 (Maddison & Maddison 2021). Eight partitioning schemes were established: one for the ITS, one for the LSU, three to represent the codon positions of the gene region *rpb*1, and three for *tef*1 gene region, which were established using the option to minimize the stop codon with Mesquite v. 3.70 (Maddison & Maddison 2021). Phylogenetic inferences were estimated with maximum likelihood in Rax-ML v. 8.2.10

(Stamatakis 2014) with a GTR + G model of nucleotide substitution. To assess branch support, 1,000 rapid bootstrap replicates were run with the GTRGAMMA model. For Bayesian posterior probability, the best evolutionary model for alignment was sought using Partition Finder v.2 (Lanfear *et al.* 2014; 2017; Frandsen *et al.* 2015). Phylogenetic analyses were performed using Mr Bayes v. 3.2.6 x64 (Huelsenbeck & 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 1,000 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 *et al.* 2014).

Results

The matrices were formed with 53 ITS sequences (605 characters), 51 LSU sequences (610 characters), 24 *rpb*1 sequences (684 characters), and 23 *tef*1 sequences (600 characters). Morphological characters, ecology, and support from 1PPB/100ML in phylogenetic analyses (concatenated and ITS) showed that *Xerocomellus piedracanteadensis* is a new species, with *X. poederi* and *X. amylosporus* as a sister group. In contrast, another species, *X. bolinii*, was identified here for the first time from Mexico, being the second record of the species previously only known from Florida and the USA, broadening the distribution of the species.

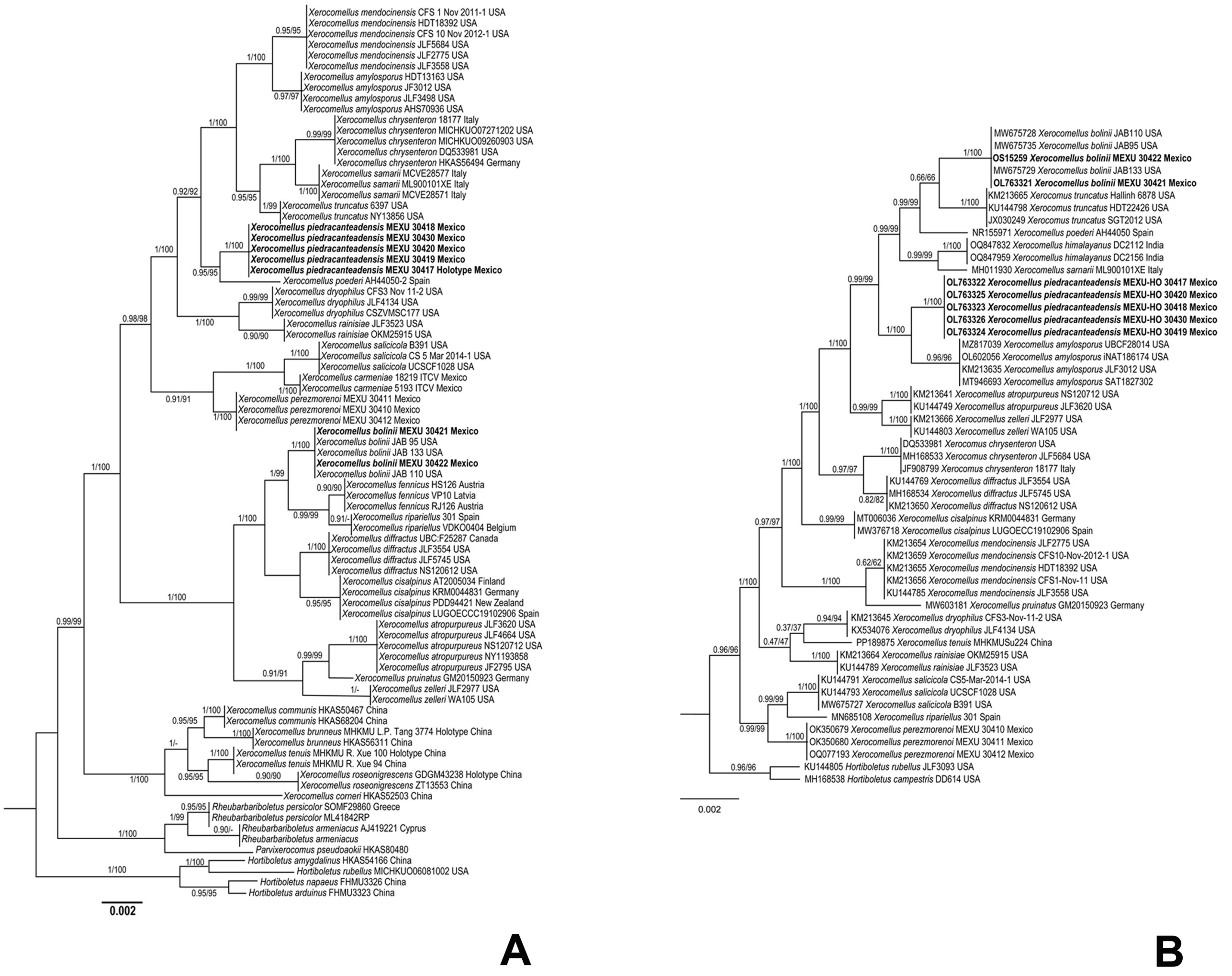

FIGURE 1. A) Phylogenetic analysis generated from the concatenate dataset (ITS, rDNA-LSU, *rpb*1, *tef*1); B) ITS Phylogenetic analysis. Trees based on maximum likelihood (values ≥70%) and Bayesian posterior probability (≥0.90) of *Xerocomellus piedracanteadensis* and *X. bolinii* relationships with other species in the genus. Boldface names represent samples sequenced in this study. As the outgroup, species of the genera *Hortiboletus, Parvixerocomus,* and *Rheubarbariboletus* were used.

Taxonomy

Xerocomellus piedracanteadensis Ayala-Vásquez, Pérez-Moreno & Martínez-Reyes, *sp. nov*. Fig. 2 Mycobank number: MB842074; GenBank OL763323 and OL763329

Etymology: *Piedra* (stone) + *canteadensis* (inclined), literally "inclined stone," refers to the type locality known in Spanish as "Piedra Canteada," which has a firefly sanctuary visited by thousands of tourists annually, located in Central Mexico.

Diagnosis:—*Xerocomellus piedracanteadensis* differs from other closely related species by brown, pale yellow to cracked vivid red pileus in age, surface stipe longitudinally striate to rivulose, basidiospores of (8)10–11–14 (15) × (3)–5 µm, elongate to cylindrical, pileipellis formed by palisoderm 300 µm thick, with terminal cells 13–55 × (8)10–23 µm, cylindrical, ovoid, subglobose to mammillate with rounded or acute apex coarsely encrusting in lower elements ringed look wall, dark brown. It grows in mixed pine-oak forests in Central Mexico.

Type:—MEXICO. Tlaxcala state, Nanacamilpa Municipality, San Felipe Hidalgo Town, Piedra Canteada Reserve, Los Chapoteaderos Place, 0542935W, 2151564N, 24 July 2021, O. Ayala-Vásquez, Pérez-Moreno J. & Martínez-Reyes M., CPM21 (MEXU-HO 30417).

Pileus 27–47 mm in diameter, broad, hemispherical to convex when young, eventually broadly convex, nearly plane, pale brown, greyish-brown (5D2,5E2,5F2), surface dry, tomentose, rivulose, at maturity cracks, areolate, showing the flesh whitish or beige to vivid red (11A6–11A8) tones. ***Hymenophore*** adnate, pores 0.3–0.8 mm, round light-yellow (4A5–4A6) bruising strongly blue-green (25A8–25B8), (24D5–24D6) when damaged, later dark blue (23E8), tubes 4–5 mm in length, concolorous with the pores. ***Context*** 6–7 mm thick, whitish, somewhat yellowish pale blue (23A4) when damaged, context stipe fibrillose, apex and middle part whitish to silky yellow (4A4–4A5); base wine-red to dark red, bluish-green (25A8–25B8), (24D5–24D6) to blue-gray (23D6) when damaged. ***Stipe*** 70–80 × 10–12 mm cylindrical, pale brown color from the middle part to the apex, the rest red (11A6–11A8) to pink-red (11A4–11A5) surface fibrillose, bluish-green (25A8–25B8) when damaged. When applying KOH in pileus and stipe in fresh there was no color change, while in hymenophore and context a brown-orange color was recorded. *Mycelium* whitish to pale yellow. Whitish pseudosclerotia measuring 20 × 10 mm, with a spongy texture, spongy ovoid, globose to subglobose with long rhizomorphs.

Basidiospores (12)13–15(16) × (4)5–6(7) µm, Q= 2.7–3 µm, (4 basidiomata, N= 120), elongate, cylindrical, some truncate with suprahilar depression, pale yellow in KOH, amyloid with Melzer solution, guttulate, think walled 0.8–1 µm. ***Basidia*** (26)28–42(47) × (8)9–12 µm, clavate, hyaline in KOH, pale yellow in Meltzer solution, with granulose content, short sterigmata. ***Basidioles*** 20–26 × 7–10 µm. ***Hymenophoral*** trama boletoid, with central hyphae 4–6 µm, cylindrical, hyaline to pale yellow, lateral hyphae 4–14 µm, cylindrical hyaline to pale yellow in KOH. ***Pleurocystidia*** 30–38 × 5–7 µm, subfusoid-ventricose, fusoid, some spheropedunculate to pyriform hyaline in KOH, pale yellow in Melzer's solution, thin-walled. ***Cheilocystidia*** 35–52 × 7–9µm, subfusoid-ventricose, fusoid, hyaline in KOH, pale yellow in Meltzer solution, thin-walled. ***Pileipellis*** formed by trichoderm 300 µm thick, with terminals cells 13–55 × (8)10–23 µm, cylindrical, ovoid, subglobose to mammillate with rounded or acute apex, yellow in KOH, orange-brown in Melzer's solution, coarsely encrusting in lower elements with brown extracellular pigments ringed look wall, dark brown. ***Caulocystidia*** 17–25 × 7–9µm, mammillate, pale yellow in KOH, yellow in Melzer's solution; ***Caulobasidia*** 17–38 × 9–10µm, 4-sterigmata, hyaline in KOH, pale yellow in Melzer's solution. ***Clamp connections*** absent.

Habitat, habit, distribution:—Solitary to scattered. Recorded from mixed forests either *Pinus- Quercus*, under *Quercus aff. crassipes*, or *Abies-Pinus-Quercus* forests, under *Q. laurina*.

Additional specimens examined:—MEXICO, Tlaxcala State, Nanacamilpa Municipality, San Felipe Hidalgo Town, Piedra Canteada, Los Chapoteaderos Place, 14 July 2021, Martínez-Reyes M-Ayala-Vásquez O., (CPM3- MEXU 30418); Piedra Canteada place, 2952 m.a.s.l., 8 September 2021, Ayala-Vásquez O., Martínez-Reyes M. (CPM74- MEXU 30419), Los Chapoteaderos Place, 8 September 2021, Ayala-Vásquez O., Martínez-Reyes M. (CPM60); El Plano Place, 25 August 2021, Ayala-Vásquez O., Martínez-Reyes M. (CPM209); El Plano Place, 15 September 2021, Ayala-Vásquez O., (CPM210); El Plano Place, 22 September 2021, Ayala-Vásquez O.,(CPM211); El Plano Place, 29 September 2021, Ayala-Vásquez O., Martínez-Reyes M. (CPM212); Rumbo a las Flores Place, 0539661 W, 2151732N, 3152 m.a.s.l., 29 September 2021, Ayala-Vásquez O., (MEXU-HO 30420), El Plano Place, 0539326 W, 2151947N, 3020 m.a.s.l., 29 September 2021, Ayala-Vásquez O., (CPM153- MEXU-HO 30430).

Notes: Some specimens were heavily parasitized by *Hypomyces microspermus* (ON231595), which covered the entire surface of the basidiomata, showing a white to pale yellow color with a cottony layer. This parasite was molecularly identified, and the corresponding sequence was deposited in the NCBI database (http://www.ncbi.nlm.nih.gov/genbank/).

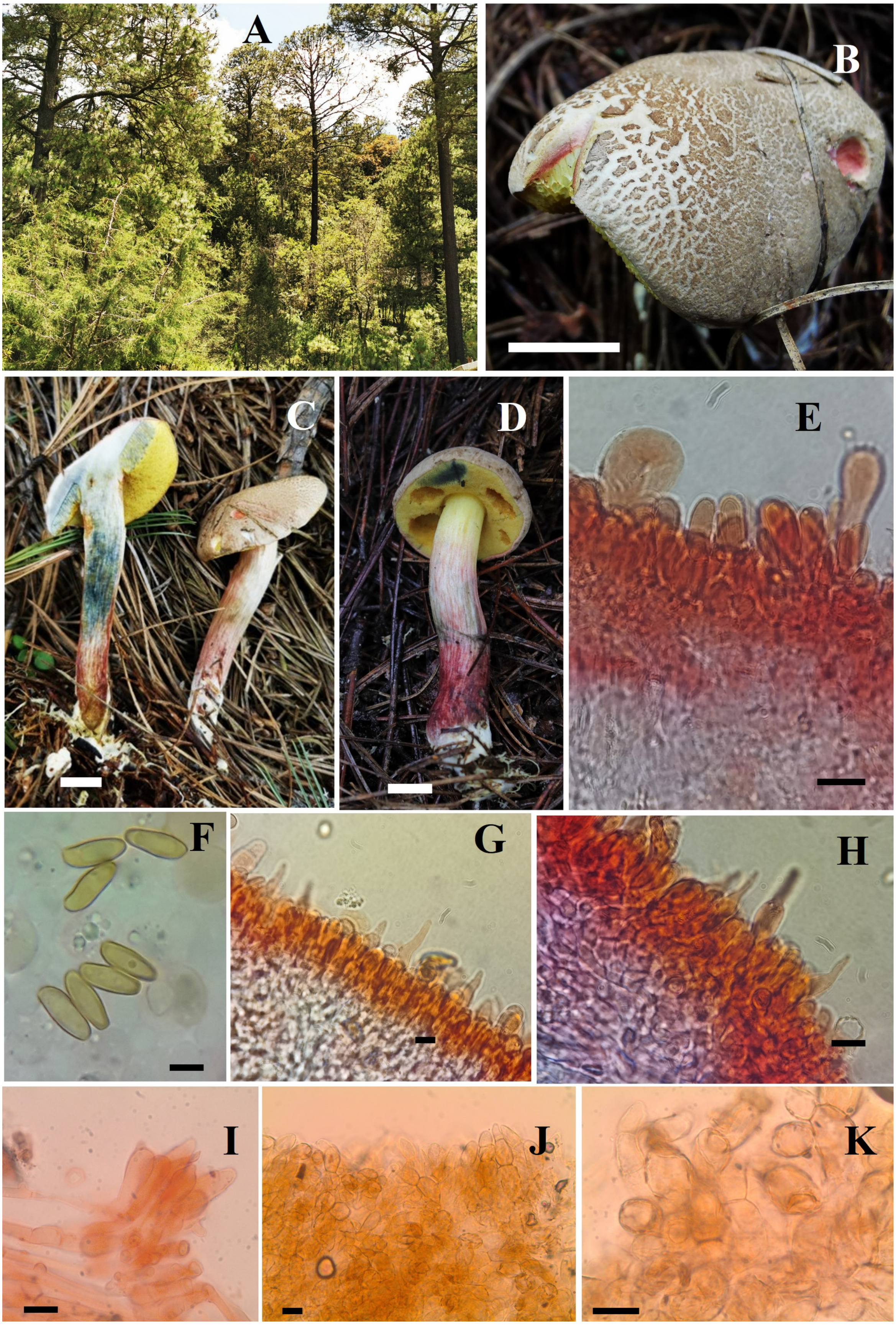

FIGURE 2. *Xerocomellus piedracanteadensis* (Holotype). **A)** Habitat of the holotype specimen showing a pine-oak mixed forest. **B)** Pileus surface. **C**) Context of basidiomata. **D**) General view showing basidiomata main characteristics (paratype MEXU-HO 30420). **E**) Basidia. **F**) Basidiospores. **G**) Pleurocystidia. **H**) Caulocystidia. **J**–K) Pileipellis. Scale bars: **B–D**: 10mm. **G–J**: 40×.

Xerocomellus bolinii J.A. Bolin, A.E. Bessette, A.R. Bessette, L.V. Kudzma, J.L. Frank & A. Farid, in Farid, Bessette, Bessette, Bolin, Kudzma, Franck & Garey, Mycosphere 12(1): 1056 (2021). Fig. 3

Mycobank number: MB 840863; GenBank OL763321 and QS15259

Xerocomellus bolinii is distinguished by a small to medium-sized basidioma. ***Pileus*** 33–80 mm, pink vivid pink to pinkish brown. ***Hymenophore*** adnexed, pores 0.5–1 mm, yellow, dull yellow angular to irregular, bluish-green to blue when cut, tubes 20–60 mm, concolorous of the pores, ***Context*** of pileus creamy white or a mixture of creamy white and yellow becoming yellow to orange in the stipe and rapidly stains blue when cutting. ***Basidiospores*** (10)13–15(16) × (4)4.5–6 µm, pale yellow, pale grayish yellow in KOH, dull yellow in Melzer's. ***Cheilocystidia*** and scattered, (36) 40–60(71) × 9–11.5 µm, fusoid-ventricose, smooth, thin walled, hyaline, yellow to ochraceous in KOH, ochraceous in Melzer's. ***Pileipellis*** formed by trichoderm with terminal hypha 30–75× 6–11µm, hyaline to pale yellow in KOH, yellow-gold in melzer solution, thin walled. The *basidiospores* of the specimens studied from Mexico are two microns larger than the specimens described from Florida, as do cystidia measuring (40)45–60 (71) × (5)6–9 (13) µm, while those in the USA measure 36–48.5 × 9–11.5 µm. The biogeographic distribution area is expanded; *X. bolinii* is distributed from East coast of the United States and in the transverse Neovolcanic axis from Mexico at altitude 40–3000 m.a.s.l., Solitary or scattered in sandy soil associated putatively with *Pinus teocote* and *P. pseudostrobus*.

Habitat, habit, distribution:—Solitary to scattered. Recorded from mixed forests with *Pinus* and *Quercus*; under *Pinus teocote* and *Pinus pseudostrobus*.

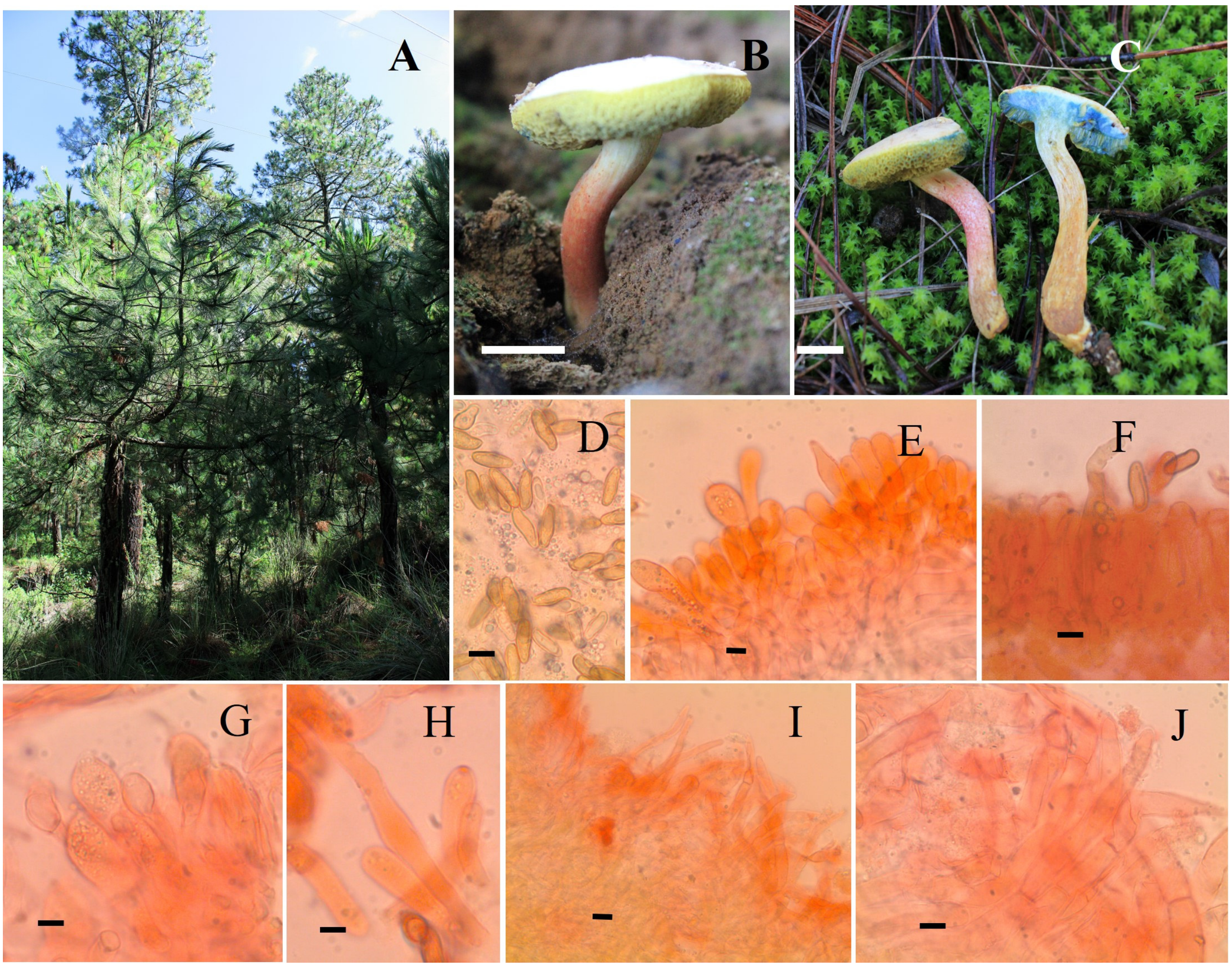

FIGURE 3. *Xerocomellus bolinii* (MEXU HO 30430). **A**) Vegetation type where some specimens were sampled, showing a mixed *Pinus*-*Quercus* forest. **B**) Basidioma in its natural habitat. **C**) Cross-section of basidioma. **D**) Basidiospores. **E**) Basidia and pleurocystidia. **F**) Cheilocystidia. **G**) Caulobasidia. **H)** Caulocystidia. **I)** Pileipellis 40×. **J**) terminal hyphal. Scale bars **B–C**:10 mm, **D–H, J**: 10µm.

Specimens examined:—MEXICO, Tlaxcala State, Nanacamilpa Municipality, San Felipe Hidalgo Town, Piedra Canteada, entrance to the Piedra Canteada Reserve; km 1 entrance to the Piedra Canteada Reserve 8 September 2021, Ayala-Vásquez O., Martínez-Reyes M. CPM55 (MEXU HO 30421); km 1 entrance to the Piedra Canteada Reserve, 15 September 2021, Ayala-Vásquez O., Martínez-Reyes M. CPM75 (MEXU HO 30422); km 1 entrance to the Piedra Canteada Reserve, 22 September 2021, Ayala-Vásquez O., Martínez-Reyes M. (CPM213).

TABLE 2. Morphological comparison of *Xerocomellus* species previously described or reported from Mexico.

Taxonomic characteristics	*X. carmeniae*	*X. perezmorenoi*	*X. truncatus*	*X. dryophillus*
Pileus	Pileus 18–32 mm, convex to flattened, deep red, vivid red, to pale brown, brown, pileus surface tomentose, areolated to cracked.	Pileus 14–50 mm, broadly convex to plane-convex, dark-brown, brown, pileus surface tomentose, areolate to cracking.	Pileus 50–110 mm, broadly convex to nearly plane, yellow-brown, brown, grey-brown to olivaceous-brown, with pinkish showing in the crack in age.	Pileus 30–110 mm, hemispheric, broadly convex to then plane in age, red-pinky, red, red-vinnaceous, surface dry, tomentose to cracked in age.
Hymenium	Pores regular to irregular, yellow to yellowish-green, tubes irregular, bruising greyish-green to greyish-blue.	Pores 0.3–1.0 mm, irregular, circular to angular light purplish, pale pink to salmon when young, yellow citrine, pastel red tones, when cut turns pale-green to olivaceous, tubes 3–9 mm, concolorous the pores.	Depressed around the stalk in age, pores 0.5–2 mm, irregular shaped, pale yellow, yellow citrine to olivaceous-yellow, bruising blue when damaged, tubes 10–15 mm deep.	Adhered, pores 0.5–1.5 mm, angular to irregular shaped, lemon-yellow, yellow to pale orange in age bruising blue quickly when damaged, tubes concolorous of pores.
Stipe	Stipe 13–34 × 0.5–10 mm, sinuous and irregular, surface.	Stipe 28–63 × 7–8 mm, attenuated, subclavate to cylindrical; yellowish, salmon pink, pastel red, surface tomentose.	Stipe 25–80 × 3–17 mm, cylindrical, tapered downward or enlarged downward, furfuraceous, yellow at the apex and medium, dull red at base, bruising blue when touched, longitudinally linear surface,	Stipe 30–120 × 70–80 mm, cylindrical, clavate at base, finely punctate surface to tomentose at base, citrus yellow to yellow, red at base.
Basidiospores	Basidiospores of 10.5–13.6 × 5.7–7.8 μm, brown, smooth, elongate, truncate, thick walled.	Basidiospores of (12–) 15–16 (–17) × 4–5 (–6) μm, fusoid to subcylindrical, to cylindrical inside view, smooth.	Basidiospores of 12–17 (–26) × (–4.2)4.5–6.5 μm, fusoid to subcylindrical, smooth, truncate-applanate, thick walled.	Basidiospores of 11.2–16 (–17.6) × 5.6–6.4 (–7.2) μm, ellipsoid to subfusoid, olivaceous, thick walled.
Pleurocystidia	Not observed	Pleurocystidia 35–60 (–68) × 5–6 μm, obclavate, cylindrical, subfusoid, subclavate, brown-yellowish, golden, to hyaline in KOH	Pleurocystidia 38–80 × 8.6 –13 μm, fusoid-ventricose, hyaline to pale yellow with visible granular content in Melzer.	Pleurocystidia 23–29.8 × 6.5–8.6 μm, fasciculate, narrowly clavate, hyaline to pale yellow in KOH.
Cheilocystidia	Not observed	Cheilocystidia 50–55 × 9–11 μm, ventricose-fusoid, thick-walled, yellowish-brown in KOH,	Cheilocystidia 40 –108 x 7.2–12 μm, fusoid-ventricose, hyaline to pale yellow with visible.	Cheilocystidia 30 –70 x 6.4–15 μm, fusoid to ventricose.
Pileipellis	Pileipellis 105-174 μm thick, composed trichoderm interwoven, with erect, clavate to fusoid terminal cells, brownish to yellowish.	Pileipellis 150–200 mm thick, palisoderm, with subfusoid, cylindrical, subclavate terminal cells, pigment borwn.	Pileipellis formed by a trichoderm, with terminal members parallel, erect or eventually sometimes depressed horizontal, 32–93×8.7–16.3 μm, ellipsoid to globose with pigment-incrusted.	Pileipellis a trichoderm with terminal cells 33–76 ×96–120 μm, subcylindrical to subclavate, pigment brown.

Key to the *Xerocomellus* in Mexico.

1a,	Pileus pink, vivid red, red to red brown	2
1b	Pileus typically pale brown, brown, black-brown to black	3
2a	Basidiomata epigeous to aberrant, pileus deep red, vivid red, hymenophore yellowish to greyish-green, basidiospores 10.5–13.6 × 5.7–7.8 µm, elongate, truncate	*X. carmeniae*
2b	Basidiomata epigeous	4
3a	Pileus brown to black brown, margin typically cream, pink to purple tones, hymenophore tubular, pores lilac, peach to salmon when young, yellow citrine at mature	*X. perezmorenoi*
3b	Pileus pale brown, greyish-brown, brown-olivaceous	5
4a	Basidiomata medium, pileus pink-red to vinaceous red, rosy red or brick-red, hymenophore pale yellow to dingy yellow or lemon-yellow, bruising blue quickly when damaged, basidiospores (11.1)12–15.7(16.1) × (5.3)5.8–6.9(7.9) µm, subellipsoid to subfusoid, associated with *Quercus agrifolia*, distributed in the Cordillera de Baja California	*X. dryophilus*
4b	Basidiomata small to medium, pileus 33– 80 mm, pink to pink-brown, with pinkish brown fibrils, context of pileus white to cream, blue when cutting, stipe of context yellow to yellow-orange rapidly stains vivid blue when exposed, basidiospores (10)13–15(16) × (4)4.5–6 µm	*X. bolinii*
5a	Basidiospores of (12)13–17(27) × 4–5(6) µm, smooth, truncate, pores yellow to yellow-olivaceous (sensu, Snell *et al.* 1959), pileus medium, pale grey-yellow, pale-brown, to olive-brown, the crack pinkish-purple, pink	*X. truncatus*
5b	Basidiospores 12–17(26) × (4)–6(7) µm, smooth, elongate, cylindrical, some truncate, pileus small to medium, pileus surface brown, greyish-brown, the crack whitish or beige to vivid red	*X. piedracanteadensis*

Discussion

Concatenated phylogenetic analysis shows that *Xerocomellus piedracanteadensis* is related to *X. poederi* However, *X. piedracanteadensis* has a medium pileus of 30–47 mm wide, pale brown, greyish-brown pileus; stipe pale brown from the middle part to the apex, the rest red to pink-red, whitish pseudosclerotia measuring 20 × 10 mm, with a spongy texture, spongy ovoid, globose to subglobose with long rhizomorphs, basidiospores (12)13–15(16) × (4)–6(7) µm. Meanwhile, *X. poederi* has a small pileus 15–55mm, pale brown (5E4), brown pinkish (9C6) to dark brown (5F8) when mature, hymenophore lemon-yellow (30A8) to yellow (4A8), deep blue (20D8–20E8) when bruised, stipe cylindrical, tapered downward, fragile often curved, red-vinnaceus (11E8)yellow (3A8) at apex, surface slightly granulose, basidiospores (10.5–)11–16(–17) × 4–5.5(–6) µm, fusiform, smooth with obtuse apex, only distributed from Spain, under *Quercus robur* (Crous *et al.* 2016). As long as the ITS analysis reveals that *X. piedracanteadensis* is close to *X. amylosporus,* but *X. amylosporus* differs by basidiomata medium to large with gastroid tendency, pileus dark brown (6F6), stipe 4–10 × 1–2 cm, stipe surface red longitudinal striations when young, to extensively brown with a red band at apex, basidiospores (11.4–)13–16.2(–18.1) × 5.2–6.5(–7.1), fusoid to subcylindrical, inequilateral, most with truncate apex, and associated with *Picea sitchensis* and *Alnus rubra* (Frank *et al.* 2020).

Xerocomellus bolinii was recently described by Farid *et al.* (2021) and is currently only known in Florida, the USA, in *Pinus-Quercus* forests. Here, we expand the distribution to Central Mexico. At the same time, in Florida, it is distributed at an altitude that ranges from sea level to 150 m; in contrast, our specimens were sampled in altitudes ranging from 2785 to 3200 m, showing that the species has a wider ecological range of altitude than previously known. The macroscopic characteristics are similar to the type species, but the Mexican specimens have longer basidiospores (12)13–15(16) × (4)4.5– 6 µm, and longer cheilocystidia (40)45–60(71) × (5)6–9 µm, while the North American specimens have basidiospores (10)12–13(14) × 4.5–6 µm and cheilocystidia 36–48.5 × 9–11.5 µm.

Xerocomellus piedracanteadensis is recorded for the first time as edible species enriching the mycocultural heritage of Mexico, the second most important center of edible wild mushrooms in the world, just after China, as reported by Pérez-Moreno *et al.* (2021a; 2021b), both studied species are consumed by some local people of San Felipe Hidalgo, and in addition, two of the coauthors corroborated the edibility by consuming them, without any adverse effect.

Conclusion

The genus *Xerocomellus* is very diverse in North America, even though the species of this genus have generally received little attention. Currently, six species of the genus are found in Mexico, two of which are reported in this work. Half of these species have been reported in the last couple of years, indicating that the studies of the genus in the country are still in their infancy. Considering the enormous diversity of *Quercus* (more than 160 species) and Pinaceae (72 taxa) in Mexico, which have been reported to be ectomycorrhizal hosts of the genus *Xerocomellus*, and the fact that half of the known species are used as a food resource by native people, this study intends to incentive both the study of the diversity of species of the genus and its biocultural relevance.

Acknowledgments

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