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## *Tricholoma colposii* (Tricholomataceae, Basidiomycota), a new edible species of matsutake fungi from Eastern Mexico with economic and biocultural importance

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### Abstract

Matsutake fungi include a complex of edible mushrooms highly prized worldwide, particularly in Japan where they are considered one of the most luxurious ingredients. These species establish ectomycorrhizal symbioses with angiosperms and gymnosperms in the Northern Hemisphere and in North America only three species are currently known. In the present contribution a fourth species of this geographical area is described, *Tricholoma colposii* sp. nov. is described from Veracruz state, in eastern Mexico based on morphological and molecular (nrITS) data. This species is characterized by middle size basidiomata, with orange brown to brown pileus and stipe, squamose when young and with appressed scales in maturity, cylindrical, fibrillose to scaly stipe, with globose to ellipsoid (4.5–)5–6 (–7) × (3–) 4–5 (–6) μm smooth spores, sporomes with sweet fruit odor and raw green bean flavour. The species grows associated with *Pinus teocote* forests. The macro- and micromorphological characters in conjunction with the nrITS-based phylogenetic analysis using Maximum Likelihood method and Bayesian inference support the proposal of this new species of Mexican matsutake. Detailed descriptions, photographs and comparisons with phenetic and phylogenetic related species are presented.

**Keywords:** biocultural importance, ectomycorrhizae, edible wild mushrooms, matsutake fungus, *Tricholomataceae*

### Introduction

*Tricholomataceae* R. Heim ex Pouzar 1983 has traditionally been considered as one of the largest families among *Agaricales*. Based on macro- and micromorphological characters, Singer (1986) considered that the family contained 98 genera. However, with the advent of molecular phylogenetics the polyphyly of *Tricholomataceae* has been demonstrated by different authors (Moncalvo *et al.* 2000, 2002; Hofstetter *et al.* 2002; Matheny *et al.* 2006; Garnica *et al.* 2007). As a consequence, most of the genera traditionally included in this family have been moved to *Cyphellaceae*, *Hydnangiaceae*, *Hygrophoraceae*, *Lachnellaceae*, *Lyophyllaceae*, *Marasmiaceae*, *Mycenaceae*, *Omphalotaceae*, *Physalacriaceae* and *Pleurotaceae* (e.g. Moncalvo *et al.* 2002; Binder *et al.* 2006; Hofstetter *et al.* 2002; Lodge *et al.* 2014; Sánchez-García *et al.* 2014). Despite the fact that a final classification of the genera once included in *Tricholomataceae* in a broad sense is far to be conclusive, currently based on morphological characters and nucleotide sequence data of ribosomal RNA (nLSU, nSSU, and ITS), and *rpb2* genes only seven genera are included in *Tricholomataceae* s. str.: *Corneriella*, *Dennisiomyces*, *Leucopaxillus*, *Pogonoloma*, *Porpoloma*, *Pseudotricholoma* and *Tricholoma* (Sánchez-García *et al.* 2014).

*Tricholoma* (Fr.) Staude is the type genus of *Tricholomataceae* originally proposed as a section by Fries (1821) and subsequently erected as a genus by Staude (1857). *Tricholoma* is a monophyletic genus of ectomycorrhizal fungi which was segregated from its ancestral clade in the late Cretaceous around 60–90 million years ago (Ryberg & Matheny 2011). Species included in the genus *Tricholoma* are distributed worldwide (Tedersoo *et al.* 2010). They form ectomycorrhizal symbiosis mainly with *Betulaceae*, *Fagaceae* and *Pinaceae* (Ryberg & Matheny 2011). They are characterized by hyaline, subglobose to oblong spores, with simple pileipellis structure, usually lacking sterile structures, including cystidia, and with clamp connections rarely present (Chrisensen & Heilman-Clausen 2012; 2013). The genus contains four subgenera: *Tricholoma*, *Sericeicutis*, *Pardincutis* and *Contextocutis*. The subgenus *Tricholoma* being the one that contains the largest number of sections including *Tricholoma*, *Genuina*, *Megatracholoma*, *Atrosquamosa*, *Terrea* and *Caligata* (Heilman-Clausen *et al.* 2017).

“Matsutake” fungi are highly prized culinary mushrooms belonging to several species included in the genus *Tricholoma* sect. *Caligata* Konrad & Maubl. ex Bon (Bon 1991) which are distributed throughout the Northern Hemisphere. Matsutake fungi have an estimated annual in-season retail market of USD\$500 million, and a wholesale price per kilogram basidiomata ranging from USD\$30 to USD\$1,000 (Pérez-Moreno *et al.* 2021a). Usually these mushrooms are host-specific: *T. matsutake*, *T. magnivelare*, *T. murrillianum* and *T. anatolicum* are mostly associated with *Pinaceae*, while *T. bakamatsutake* and *T. fulvocastaneum* grow mainly in *Fagaceae* forests (Murata *et al.* 2013). Most of the species which belong to this complex are quasiendemic, for example: i) *T. matsutake* grows in Eastern Asian countries, mainly China, Japan and Korea, and also in Scandinavian countries; ii) *T. fulvocastaneum* inhabits East and Southeast Asia; iii) *T. anatolicum* is native from the Mediterranean area; iv) *T. magnivelare* is distributed in Eastern North America; and v) *T. murrillianum* grows in western North America (Kytoivuori 1989; Bergius & Danell 2000; Sanmee *et al.* 2007; Dođan & Akata 2011; Murata *et al.* 2013; Trudell *et al.* 2017). Currently, in Mexico only one species has been reported using both molecular and morphological characteristics: *T. mesoamericanum*. Trudell *et al.* (2017) described this species based only on one ITS sequence of a specimen from Ixtlán, Oaxaca state, in southwestern Mexico and additionally they recorded this species based only in morphological characters of specimens from different parts of the country.

In this contribution based on a detailed macro- and micromorphological and molecular (ITS) study we describe *T. colposii*, a new species of matsutake fungi growing in *Pinus teocote* Schiede ex Schltdl. forests in Eastern Mexico, an edible species with great biocultural and economic importance.

## Materials & methods

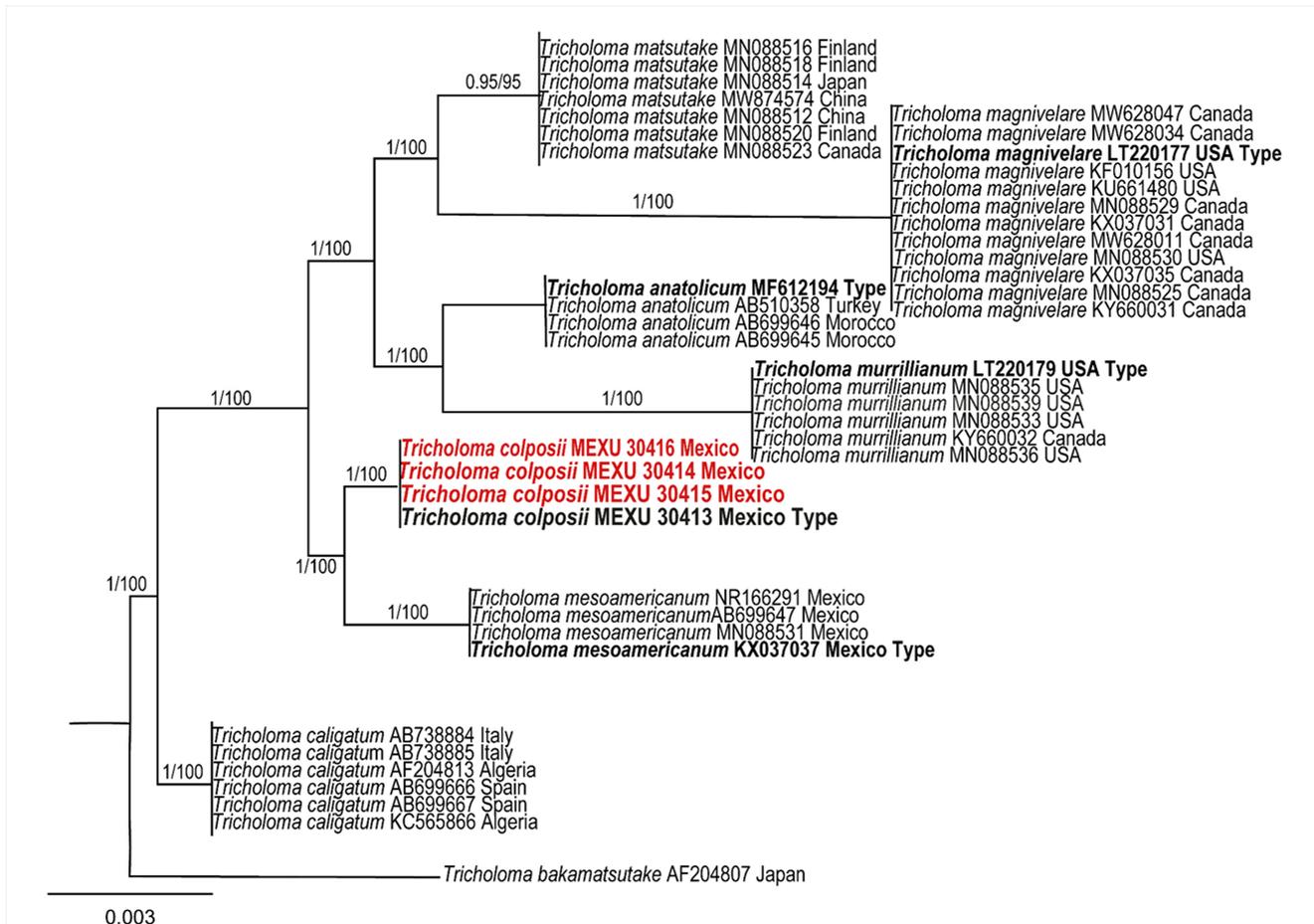
### *Field and laboratory work*

Basidiomata were collected in the Cofre de Perote region, state of Veracruz, Mexico, during the months of August to October 2018 and 2021. The main vegetation type in the region are *Pinus-Quercus* forests dominated by *Pinus teocote*, *P. montezumae* Lamb, *P. pseudostrobus* Lindl., *Q. laurina* Humb et Bonpl., and *Quercus* spp. Protocols for sampling macrofungi were followed (Lodge *et al.* 2004). All of the studied specimens were collected growing under *P. teocote* natural forests. Cuts were made in herborized specimens and temporary preparations were made using 5% KOH, congo red, and Melzer’s reagent. The size of the microcharacters such as basidia, and basidiospores were determined by measuring at least 35 elements each. The next abbreviations are used: Q refers to the length/wide ratio. The studied material was deposited in herbarium “Colpos” at the Colegio de Postgraduados, Campus Montecillo, in Texcoco, Mexico with replicates in the mycological herbarium (MEXU-UNAM) of the Universidad Nacional Autónoma de México located in Mexico City, Mexico.

### *DNA extraction, PCR amplification and sequencing*

Genomic DNA was obtained with CTAB (Martínez-González *et al.* 2017) from 2–3 mg of dry tissue. DNA quantification was performed with Nanodrop (Thermo, USA). The Internal Transcribed Spacer (ITS) region was amplified with the primer ITS5 (GGAAGTAAAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White *et al.* 1990). The PCR reaction contained the following: enzyme buffer 1x, 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, USA), with a final volume of 15 µL. The amplification program was run as follows: denaturalization at 95°C for 4 min, 35

cycles of denaturalization at 95°C for 1 min, annealing at 57°C for 1 min, polymerization at 72°C for 2 min, and final elongation at 72°C for 10 min. All PCR reactions were carried out in a Peltier Thermal Cycler PTC-200 (BIORAD, Mexico). The PCR products were verified by agarose gel electrophoresis. The gels were run for 1 h at 95 V cm<sup>3</sup> 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, Germany). The amplified products were purified with the ExoSAP Purification kit (Affymetrix, USA), following the manufacturer's instructions. They were quantified and prepared for the sequence reaction using a BigDye Terminator v. 3.1 (Applied Biosystems, USA). These products were sequenced in both directions with an Applied Biosystem model 3730XL (Applied BioSystems, USA). The sequences obtained were compared with the original chromatograms to detect and correct possible reading errors. The sequences of both strains of each sample 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).



**FIGURE 1.** Phylogram of Bayesian inference (BI) tree from the ITS sequence data of 44 specimens. *Tricholoma bakamatsutake* was used as the outgroup. The values above branches represent Bayesian posterior probabilities (PP, left) and bootstrap values (LP, right) for maximum likelihood, respectively. The scale bar represents the expected number of nucleotide substitutions per site. Sequences obtained from this study are in red. Type species are in bold.

### Phylogenetic analysis

In order to study phylogenetic relationships, our newly produced sequences of four individuals of *Tricholoma* were added to reference sequences of ITS (Table 1) deposited in the NCBI database (<http://www.ncbi.nlm.nih.gov/genbank/>). The ITS region was 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 matrix was composed of 44 taxa (668 characters). *Tricholoma bakamatsutake* (access number AF204807) was used as the outgroup. 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, 10000 nonparametric rapid bootstrap pseudoreplicates were run with the GTRCAT 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  $\leq 0.1$ ) 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), and they edited in Adobe Illustrator vCS4 (Adobe Systems, Inc., San Jose, CA).

## Results

### Phylogenetic results

Molecularly, the BLASTn results using the ITS (668 bp) sequence showed that the closest matches of *Tricholoma colposii* had a similarity of 94% with *T. mesoamericanum*. Sequences produced in this study of *T. colposii* were deposited in GenBank (Table 1). The two simultaneous Bayesian runs continued until the convergence parameters were met, and the standard deviation fell below 0.001 after 10 million generations. No significant changes in tree topology trace or cumulative Split frequencies of selected nodes were observed after about 0.2 million generations, so the first 2,500,000 sampled trees (25%) were discarded as burn-in. Both the Bayesian analyses and Maximum Likelihood (Fig. 1) recovered *T. colposii* supporting the existence of one new taxon distinctive from related species of *Tricholoma*.

## Taxonomy

*Tricholoma colposii* J. Pérez-Moreno, M. Martínez-Reyes M. & O. Ayala-Vásquez O. *sp. nov.* Figure 2.  
Mycobank no. MB843068; GenBank. OM732329

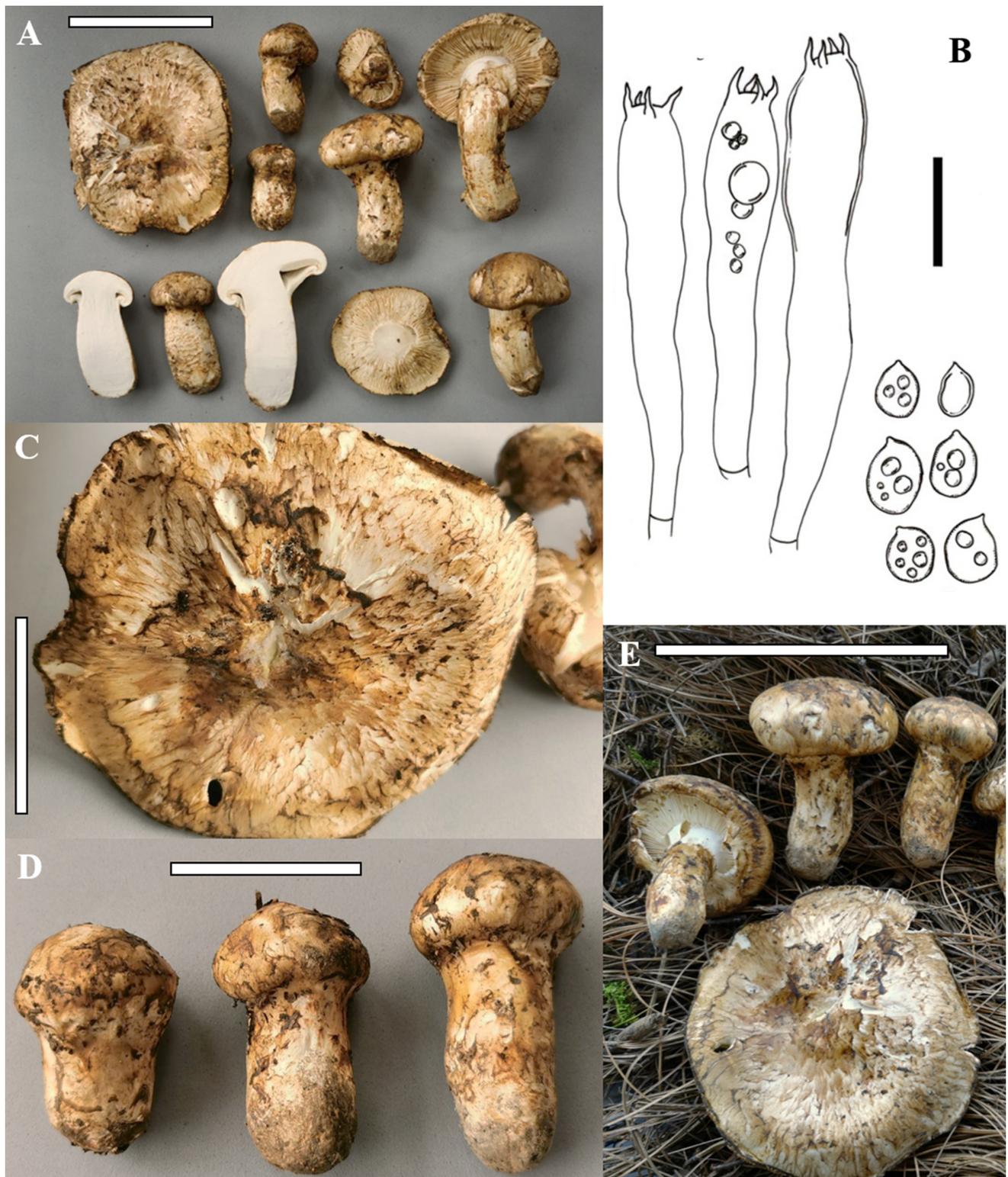
**Diagnosis:**—It is characterized by middle size basidiomata, with orange brown to brown pileus and stipe, squamose when young and with appressed scales in maturity, cylindrical, fibrillose to scaly stipe, with globose to ellipsoid (4.5–)5–6 (–7)  $\times$  (3–) 4–5 (–6)  $\mu\text{m}$  smooth spores, sweet fruit odor and raw green bean flavour sporomes. It associated with *Pinus teocote* in eastern Mexico forests. The macro- and micromorphological characters in conjunction with the nrITS-based phylogenetic analysis support the proposal of this new species of matsutake fungus.

**Holotype:**—MEXICO. Veracruz state, Parque Nacional Cofre de Perote, 1 September 2021, Flores Armas L., (CP161, MEXU 30413). GenBank [ITS]: OM732326.

**Etymology:**—“*colposii*” refers to Colegio de Postgraduados, commonly abbreviated as “Colpos”, which is an Agricultural teaching and research institution in Mexico which has funded research related with edible ectomycorrhizal mushrooms during more than 30 years.

**Description:**—*Pileus* 31–85 mm in diameter, broadly convex, convex to plane with a slight central depression at maturity, orange-brown (6C6) to brown (6D6–6D7), showing some beige to whitish tones, surface light brown, orange-brown radial, triangular to circular fibrils, central fibril slightly darker than margin, dry, margin fully adherent to partial veil when young, fibrillose texture fully covering hymenium, torn, with remnants of the veil at maturity. *Hymenophore* adhered, lamellae close, sinuate, whitish when young, beige when mature with pale brown tones. *Context* 6–8 mm thick, whitish, solid, without change in color when cut. *Stipe* 25–103  $\times$  15–22 mm, cylindrical to subclavate, surface with fibrils covering the base to the middle, dry, with a satiny appearance orange-brown (6C6) to brown (6D6–6D7), revealing some beige to whitish shades, completely white apex, very marked irregular ring in the middle part. *Basal mycelium* whitish. *Odor* sweet, fruity. *Taste* raw green bean flavor (*Phaseolus vulgaris* L.).

*Basidiospores* (4.5–)5–6 (–7)  $\times$  (3–) 4–5 (–6)  $\mu\text{m}$ ,  $Q=1.1$ –1.8  $\mu\text{m}$ , ( $n=50$ ), ellipsoid to subglobose, hyaline in KOH, nonamyloid, pale brown in Melzer, thick-walled, guttulate with or without visible suprahilar depression. *Crassospores* observed, thick-walled. *Basidia* 32–40 (–48)  $\times$  (6–) 7–8  $\mu\text{m}$ , 4-sterigmata, narrowly clavate, long sterigmata 3–4  $\times$  1–1.2  $\mu\text{m}$ , with granular content. *Basidioles* 30–35  $\times$  7–8  $\mu\text{m}$ . *Hymenial trama* parallel, composed of cylindrical hyphae (3–) 4–10  $\mu\text{m}$ , hyaline in KOH, thick-walled 1  $\mu\text{m}$ . *Stipitipellis* formed by tubular hyphae 5–8  $\mu\text{m}$ , thick-walled 1–1.2  $\mu\text{m}$  hyaline in KOH. *Pileipellis* formed by a trichoderm of intertwined hyphae in a radial arrangement, terminal hyphae 20–58 (–75)  $\times$  (6–) 7–8  $\mu\text{m}$ , cylindrical, clavate, subglobose to pyriform, hyaline to pale brown in KOH, brown in melzer, thick-walled.



**FIGURE 2.** *Tricholoma colposii* (MEXU 30413, holotype). **A.** Basidiomata in different stages. **B.** Basidia and basidiospores. **C.** Close-up of orange-brown to brown fibrillose to scaly pileipellis. **D.** Grade 1 basidiomata which are the most expensive. **E.** Basidiomata in a *Pinus teocote* forest, its natural habitat. Scale Bar: **A and E**= 100 mm, **B** = 10 $\mu$ m; **C and D**= 50 mm.

**Notes:**—*Tricholoma colposii* differs from its closest relative *T. mesoamericanum* by the white and smooth pileus surface in young specimens and a stipe with yellow or brown spots all over, and basidiospores 4.5–6.5  $\times$  3.5–4.5  $\mu$ m, while *T. colposii* has orange-brown basidiomata, with scales or fibrils from the immature stage, fibrillose to scaly cylindrical equal stipe, and basidiospores 5–6 (–7)  $\times$  4–5 (–6)  $\mu$ m. Molecularly both species are monophyletic but located in different clades clearly separated (Fig. 1).

**TABLE 1.** *Tricholoma* taxa, geographic location, references and GenBank accession numbers of ITS sequences used in the molecular analysis. Sequences of type species are in bold.

<b>Taxon</b>	<b>Voucher ID</b>	<b>Location</b>	<b>ITS accession</b>	<b>Reference</b>
<i>T. anatolicum</i>	MC1	Morocco	AB699645	Ota <i>et al.</i> 2012
<i>T. anatolicum</i>	TM-5	Morocco	AB699646	Ota <i>et al.</i> 2012
<i>T. anatolicum</i>	Tur1	Turkey	AB510358	Direct Submission
<b><i>T. anatolicum</i></b>	HD1058 Type	<b>Turkey</b>	<b>MF612194</b>	<b>Intini <i>et al.</i> 2003</b>
<i>T. bakamatsutake</i>	Tb 1	Japan	AF204807	Kikuchi <i>et al.</i> 2000
<i>T. caligatum</i>	Tc 1	Algeria	AF204813	Kikuchi <i>et al.</i> 2000
<i>T. caligatum</i>	SCM:B-5116	Spain	AB699667	Ota <i>et al.</i> 2012
<i>T. caligatum</i>	TFM:M-R106	Italy	AB738884	Murata <i>et al.</i> 2013
<i>T. caligatum</i>	TFM:M-R107	Italy	AB738885	Murata <i>et al.</i> 2013
<i>T. caligatum</i>	CM030	Algeria	KC565866	Benazza-Bouregba <i>et al.</i> 2016
<i>T. caligatum</i>	SCM:B-4194	Spain	AB699666	Ota <i>et al.</i> 2012
<i>T. colposii</i>	MEXU 30416	Mexico	OM732329	This study
<b><i>T. colposii</i></b>	<b>MEXU30413 Type</b>	<b>Mexico</b>	<b>OM732326</b>	<b>This study</b>
<i>T. colposii</i>	MEXU 30414	Mexico	OM732327	This study
<i>T. colposii</i>	MEXU 30415	Mexico	OM732328	This study
<i>T. magnivelare</i>	Arora13201	USA	KF010156	Trudell <i>et al.</i> 2017
<i>T. magnivelare</i>	CSU CLO1493	USA	KU661480	Trudell <i>et al.</i> 2017
<b><i>T. magnivelare</i></b>	<b>NYSf2421 Type</b>	<b>USA</b>	<b>LT220177</b>	<b>Trudell <i>et al.</i> 2017</b>
<i>T. magnivelare</i>	JX-2017	Canada	KX037031	Trudell <i>et al.</i> 2017
<i>T. magnivelare</i>	MICH:184263	USA	MN088530	Sandor <i>et al.</i> 2020
<i>T. magnivelare</i>	MQ20-HL0664-QFB31076	Canada	MW628011	Direct Submission
<i>T. magnivelare</i>	MQ20-HL1613-QFB32585	Canada	MW628034	Direct Submission
<i>T. magnivelare</i>	NFLD3	Canada	KX037035	Trudell <i>et al.</i> 2017
<i>T. magnivelare</i>	NS-16-307-01	Canada	KY660031	Trudell <i>et al.</i> 2017
<i>T. magnivelare</i>	NS-16-307-01	Canada	MN088529	Sandor <i>et al.</i> 2020
<i>T. magnivelare</i>	MQ20-HRL1887-QFB32638	Canada	MW628047	Direct Submission
<i>T. magnivelare</i>	TmNFLD3	Canada	MN088525	Sandor <i>et al.</i> 2020
<i>T. matsutake</i>	07JL10	China	MN088512	Sandor <i>et al.</i> 2020
<i>T. matsutake</i>	JA2	Japan	MN088514	Sandor <i>et al.</i> 2020
<i>T. matsutake</i>	N1	Finland	MN088516	Sandor <i>et al.</i> 2020
<i>T. matsutake</i>	S21	Finland	MN088518	Sandor <i>et al.</i> 2020
<i>T. matsutake</i>	S40	Finland	MN088520	Sandor <i>et al.</i> 2020
<i>T. matsutake</i>	TmNFLD1	Canada	MN088523	Sandor <i>et al.</i> 2020
<i>T. matsutake</i>	YMF5.0154	China	MW874574	Zhang <i>et al.</i> 2021
<b><i>T. mesoamericanum</i></b>	<b>FCME21585 Type</b>	<b>Mexico</b>	<b>KX037037</b>	<b>Trudell <i>et al.</i> 2017</b>
<i>T. mesoamericanum</i>	FCME 21585	Mexico	NR_166291	Direct Submission
<i>T. mesoamericanum</i>	FCME 21585	Mexico	MN088531	Direct Submission
<i>T. mesoamericanum</i>	MX1	Mexico	AB699647	Ota <i>et al.</i> 2012
<i>T. murrillianum</i>	MICH:165484	USA	MN088535	Sandor <i>et al.</i> 2020
<i>T. murrillianum</i>	MICH:165500	USA	MN088536	Sandor <i>et al.</i> 2020
<i>T. murrillianum</i>	MICH:42429	USA	MN088539	Sandor <i>et al.</i> 2020
<i>T. murrillianum</i>	SAT-16-319-01	Canada	KY660032	Trudell <i>et al.</i> 2017
<i>T. murrillianum</i>	WTU:F-012635	USA	MN088533	Sandor <i>et al.</i> 2020
<b><i>T. murrillianum</i></b>	<b>NY586560 type</b>	<b>USA</b>	<b>LT220179</b>	<b>Trudell <i>et al.</i> 2017</b>

**TABLE 2.** Table of comparison American *Tricholoma* species.

Character	<i>T. colposii</i>	<i>T. mesoamericanum</i>	<i>T. magnivelare</i>	<i>T. murrillianum</i>
Pileus	Pileus 31–85 mm in diam, broadly convex, convex to plane-convex to plane with a slight central depression at maturity, orange-brown to brown, showing some beige to whitish tones, surface light brown, orange-brown radial, triangular to circular fibrils.	Pileus 40–165 mm diam, convex or occasionally broadly subumbonate to plano-convex, becoming depressed at center in older specimens; surface white and smooth in young specimens, fibrils more evident in age, pale yellow to brown, distinctly brown all over in older specimens, dry to viscid.	Pileus 50–200 mm diam, convex when young, expanding to plano-convex, surface smooth to scaly and dry, at first beige becoming light brown radial fibrils.	Pileus 50–210 mm, convex or broadly umbonate when young, expanding to plano-convex, surface dry to subviscid, shining to dull when dry, smooth, or becoming slightly fibrillose, at first whitish, disc and fibrils becoming ochraceous to brownish with age.
Odor and taste	Sweet citrus fruit odor and raw green bean flavor.	Odor fragrant, sweet with somewhat spicy character, taste mild to somewhat sweet.	Strong mushroom, spicy (cinnamon), floral notes and undertones of citrus, and bitter almond.	Odor strong, fragrant, and distinctive; taste spicy.
Lamellae	Lamellae close, sinuate, whitish when young, beige when mature with pale brown tones.	Lamellae crowded to very crowded, adnate to sinuate, white, spotted brown in age, with concolorous, entire edge.	Lamellae adnexed to sinuate, close to crowded, whitish to pale cream becoming spotted orange to red-brown in age or when bruised.	Lamellae adhered, close; short-lamellae, white to ivory, sometimes developing brown or reddish-brown stains.
Stipe	Stipe 25–103 × 15–22 mm, cylindrical to subclavate, surface with fibrils covering the base to the middle, dry, with a satiny appearance orange-brown to brown, revealing some beige to whitish shades.	Stipe 40–175 × 15–40 mm, cylindrical, tapered toward the base; surface dry or slightly viscid, white to pale yellow.	Stipe 40–250 × 20–50 mm, cylindrical, tapered toward base; surface dry or slightly viscid, white above annulus, concolorous with cap below.	Stipe 60–150 × 20–40 mm, cylindrical, tapered towards the base; surface white and furfureaceous above the annulus, concolorous with pileus below the annulus, becoming orange to red-brown with age or where bruised.
Basidiospores	Basidiospores (4.5–)5–6 (–7) × (3–) 4–5 (–6) µm, ellipsoid to subglobose, hyaline in KOH, nonamyloid, thick-walled, guttulate with or without apiculus.	Basidiospores white in mass, smooth, nonamyloid, 4.5–6.5 × 3.5–4.5 µm,	Basidiospores 5–7.5 × 3.5–5.5 µm, white in mass, smooth, nonamyloid, ellipsoidal to subglobose.	Basidiospores 5–7.5 × 3.5–5.5 µm; ellipsoid to nearly subglobose, with a small apiculus; smooth; hyaline in KOH
Basidia	Basidia 32– 40 (–48) × (6–)7–8 µm, 4-sterigmata, narrowly clavate, with granular content.	Basidia 22–38 × 4.5–6.5 µm, narrowly clavate, mostly 2–4 sterigmata,	Basidia 32–40 × 6.5–7.5 µm, clavate, 4-spored.	Basidia 30–43 × 6.0–7.0 µm, clavate, 4-spored.
Pileipellis	Pileipellis a trichoderm of intertwined hyphae in a radial arrangement, terminal hyphae 20–58(–75) × (6–) 7–8 µm, cylindrical, clavate, subglobose to pyriform, hyaline to pale brown.	Pileipellis a cutis, in areas transitional to an ixocutis, composed of hyphae 5–11 µm wide, mostly hyaline, some with pale brown intracellular pigment.	Pileipellis a cutis, composed of hyphae 3–10 µm, hyaline.	Pileipellis a cutis, elements 5–7.5 µm wide, smooth, hyaline to yellowish in KOH.
Habit, habitat and distribution	Solitary to scattered. Known from the Cofre de Perote, from Mexico, growing in mixed forest <i>Pinus-Quercus</i> , formed mycorrhizae with <i>Pinus teocote</i> .	Solitary to gregarious, in montane 2000–3000 m elevation in mixed <i>Pinus-Quercus</i> forests from Mexico.	Solitary, scattered, or gregarious, under <i>Pinus contorta</i> , <i>P. banksiana</i> , <i>P. resinosa</i> , <i>P. rigida</i> , from eastern North American, and Canada.	Solitary to gregarious, sometimes caespitose, Mycorrhizal, primarily with <i>Pinus</i> , it is distributed from western North America to the Rocky Mountains westward.
Phenology	August to October	August to October	September to October	November

**Habitat, habit, distribution:**—Solitary to disperse. Known from the Cofre de Perote National Park, from Mexico, growing under *Pinus teocote* forests, from the end of August to the end of October, having a late short at the end of the rainy season phenology.

**Specimens examined:**—MEXICO, Veracruz state, Parque Nacional Cofre de Perote National Park, 15 August 2018, L. Flores-Armas (Colpos 25), 25 August 2021, Mycoredes, (CPM162, MEXU 30414), 2 September 2021, Mycoredes, (CPM163, MEXU 30415). MEXICO, Parque Nacional Cofre de Perote, 2 October 2021, Mycoredes, (CP45, MEXU 30416).

## Discussion

*Tricholoma colposii* is presented as a new species based on molecular, morphological, and geographical information. Thirty-three years ago, Villareal and Pérez-Moreno (1989) based only on morphological characters identified from the study area a matsutake fungus species as *T. magnivelare*, which was a correct name according to the prevailing knowledge at that time and they documented a great biocultural and economic relevance for this taxon mainly as a genetic resource exported to Japan. However, with the current advent of molecular techniques and the consequent rearrangement of North American species of matsutake proposed by Trudell *et al.* (2017), a re-examination of fresh specimens from the area was necessary. This scrutiny conducted to the raising of the new species described in this work based on detailed morphological, molecular and phylogenetic analyses. *Tricholoma colposii* is morphologically and phylogenetically related to those of the “matsutake group”. All the species in this group share morphological and microscopical features and sometimes show subtle phenetic variations (Trudell *et al.* 2017). Nevertheless, the pileus color and surface, stipe surface and form, distribution and tree host species help to segregate the species. *Tricholoma colposii* is nested in a well-supported clade along with *T. mesoamericanum* but in distinctive different branches. Both species occur in Mexican temperate forests associated with *Pinus*, although *T. mesoamericanum* shows a paler coloration in young specimens, brownish pileus when mature with thinner pileal scales and the absence of dextrinoid crassosporous.

*Tricholoma magnivelare* is a related species, but it can be segregated from *T. colposii* due to the paler coloration of the basidiomata, paler lamellae and its distribution in eastern USA and Canada in north America where is associated with *Pinus banksiana*, *P. resinosa*, *P. rigida*, and *Tsuga canadensis*. *Tricholoma murrilianum* is found in western USA and it has whitish basidiomata, less scaly pileus and is associated with *Abies*, *Arctostaphylos*, *Notholiticarpus Pinus*, *Pseudotsuga* and *Tsuga*. Other related species are *T. anatolicum*, a turkish species characterized by the cedar-like odor, the presence of cystidia and the association with *Cedrus* (Dođan & Akata 2011). *Tricholoma matsutake* have a similar pleasant odor, but differs from *T. colposii* mainly by the geographic distribution in southeastern Asia and Scandinavia (Bergius & Danell, 2000). *Tricholoma caligatum* is distributed in Africa and the Mediterranean, and is characterized by the smaller pileus (less than 100 mm), bitter taste and blackish tones (Kytövuori 1989).

The discovery of this new edible species hopes to stimulate deeper studies related to its ecology and sustainable management. With the description of *T. colposii*, now four species from the Matsutake group are known from North America. Furthermore, the high diversity of host trees such as *Pinus* and *Quercus* in Mexico (Gernandt & Pérez-de la Rosa 2014; Valencia 2004), may contain some undescribed *Tricholoma* species. This study encourages to continue with the research related to the Mexican ectomycorrhizal wild edible fungi.

*T. colposii* has a great biocultural, social and economic importance in the area where it grows. It receives different names, in the different localities where it inhabits, e.g. in the localities of Rancho Nuevo and Escobillo which belong to the Municipality of Perote is known as “perfumado” (scented) and “hongo apestoso” (stinky mushroom), respectively. And in the community El Llanillo, Municipality of Las Vigas de Ramírez it is known as “hongo blanco” (white mushroom) or “taquechi” (referring to the nickname of the local gatherer who has the deepest knowledge of the specific sites where this species grows). Interestingly, the word Takeshi is a common male name of Japanese origin that means military or warrior. The commercialization of *T. colposii* is conducted directly from the gatherers to Japanese buyers, who usually are brokers or Japanese consumers. It is not possible to buy this species in the local markets as it happens for a number of other edible wild mushrooms. The price of this mushrooms goes from MX\$600 to MX\$800 (USD\$30 to 40) per Kg compared to MX\$3 to MX\$4 which is the cost of species of *Amanita* sect. *Caesarea*, *Ramaria* spp., *Suillus* spp. and *Laccaria* spp. which grow abundantly in the area and are frequently commercialized in Mexican markets (Pérez-Moreno *et al.* 2008). Despite the fact that the prizes of *T. colposii* might seem low compared with other matsutake fungi, for example *T. matsutake* whose price goes from USD\$30 to USD\$1,000 per Kg in

international markets (Pérez-Moreno *et al.* 2021a), it has a high economic importance in the area where it grows, where the daily wage goes from MX\$150 to MX\$170 (USD\$7.50 to USD\$8.00). As a consequence, the sites where *T. colposii* grow are usually kept in secret by families, even among local people, due to its high local value. However, is important to highlight the importance of dissemination of sustainable harvest among local gatherers of this precious non-timber forest product in order to avoid its destruction or production decrease, as it has happened for other valuable ectomycorrhizal fungi, for example *Thelephora ganbajun* or *Tuber indicum* in some parts of China (Pérez-Moreno *et al.* 2021b).

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