Revista Mexicana de Biodiversidad

Revista Mexicana de Biodiversidad 93 (2022): e934893

Taxonomy and systematics

Tuber caryophilum, a new truffle species growing in *Carya illinoinensis* orchards

Tuber caryophilum, *una especie nueva de trufa creciendo en huertos de* Carya illinoinensis

Judith A. Sánchez-Ledesma [a], Gonzalo Guevara-Guerrero [b, *], Roberto Garibay-Orijel [c], Rodolfo Ángeles-Argáiz [c], Verónica Ávila-Rodríguez [d], Jesús G. Arreola-Ávila [a], Violeta Carrasco-Hernández [e], Amparo Borja-de la Rosa [e], Fabián González-García [a]

[a] *Universidad Autónoma Chapingo, Unidad Regional Universitaria de Zonas Áridas, Km. 40, Carretera Gómez Palacio-Chihuahua, 35230 Bermejillo, Durango, Mexico*

[b] *Instituto Tecnológico de Cd. Victoria, Av. Portes Gil 1301 Pte., 87010 Cd. Victoria, Tamaulipas, Mexico*

[c] *Universidad Nacional Autónoma de México, Instituto de Biología, Circuito exterior s/n, Ciudad Universitaria, Coyoacán, 04510 Ciudad de México, Mexico*

[d] *Universidad Juárez del Estado de Durango, Facultad de Ciencias Biológicas, Av. Universidad s/n, Fracc. Philadelphia, 35010 Gómez Palacio, Durango, Mexico*

[e] *Universidad Autónoma Chapingo, Km 36.5 Carretera México-Texcoco, 56230 Chapingo, Estado de México, Mexico*

*Corresponding author: guevaragg@hotmail.com (G. Guevara-Guerrero)

Received: 15 December 2021; accepted: 4 July 2022

Abstract

Tuber is a genus of ectomycorrhizal fungi with an important diversity of species associated with hosts in Juglandaceae. *Tuber caryophilum* is proposed as a new species based on ecological, morphological and phylogenetic characters of 2 ribosomal markers (ITS and LSU). This species is characterized by forming ectomycorrhizae on the roots of *Carya illinoinensis* (nogal pecanero) in the Comarca Lagunera of Coahuila and Chihuahua, Mexico and by exhibiting 18-48 × 10-27 µm echinulate ascospores. *Tuber caryophilum* belongs to the Rufum clade and is the sister species of *Tuber theleascum*, a species reported in northern Mexico associated with *Quercus canbyi* and *Q. polymorpha.* These 2 truffles belong to a clade from the southern USA and northern Mexico with taxa associated to *Quercus* and *Carya*, such as *Tuber lyonii.*

Keywords: Diversity; Ectomycorrhizal fungi; Hypogeous fungi; Pecan

Resumen

Tuber es un género de hongos ectomicorrízicos con una importante diversidad de especies asociada a hospederos en Junglandaceae. *Tuber caryophilum* es propuesta como una especie nueva basada en caracteres ecológicos, morfológicos

ISSN versión electrónica: 2007-8706; Universidad Nacional Autónoma de México, Instituto de Biología. https://doi.org/10.22201/ib.20078706e.2022.93.4893

y filogenéticos de 2 marcadores ribosomales (ITS y LSU). Esta especie se caracteriza por formar ectomicorrizas en las raíces de *Carya illinoinensis* (nogal pecanero) en la Comarca Lagunera de Coahuila y en Chihuahua, México y por presentar ascosporas equinuladas de 18-48 × 10-27 µm. *Tuber caryophilum* pertenece al clado Rufum y es la especie hermana de *Tuber theleascum*, una especie descrita del norte de México asociada con *Quercus canbyi* y *Q. polymorpha.* Estas 2 especies de trufas pertenecen a un clado del sur de EUA y norte de México con taxones asociados a *Quercus* y *Carya* como *Tuber lyonii.*

Palabras clave: Diversidad; Hongos ectomicorrizógenos; Hongos hipogeos; Nogal pecanero

Introduction

Species of the genus *Tuber* P. Micheli ex F.H. Wigg belong to the family Tuberaceae (Pezizales) and form ectomycorrhizae with many forest tree species (Bonito et al., 2011; Li et al., 2018). These species are ecologically and economically important (Guevara et al., 2013; Neri-Luna et al., 2012). Approximately 86 species of *Tuber* are known worldwide (Guevara et al., 2013; Kirk et al., 2008). They grow in mycorrhizal symbiotic association with gymnosperm and angiosperm trees. Thirty-eight species of *Tuber* have been described in North America (Guevara et al., 2013). The diversity of the genus in Mexico is expected to be high because this region is a center of diversification of *Quercus* Kappelle, Maarten and *Pinus* Linneo, 2 of the main ectomycorrhizal hosts of *Tuber.* Knowledge of *Tuber* in Mexico has recently expanded. For example, new species belonging to the Maculatum clade, such as *Tuber castilloi* Guevara, Bonito & Trappe, *T. guevarai* Bonito & Trappe (Guevara et al., 2013), *Tuber mexiusanum* Guevara, Bonito & Trappe (Guevara et al., 2013), *T. mixtecorum* J. García, Ayala Vázquez & de la Fuente (García-Jiménez et al., 2021) and *T. theleascum* M. Leonardi, A. Paz, G. Guevara & Pacioni (Leonardi et al., 2019) associated with *Quercus* spp., have been described in northeastern Mexico. *Tuber incognitum* and *T. anniae* belonging to the *Puberulum* clade have also been recorded in association with *Quercus* spp and *Pinus montezumae* in central Mexico (Piña-Páez et al., 2018). In association with trees of forest interest in Mexican ecosystems, *T. guzmanii*, *T. separans*, and *T. pseudoseparans* have also been found (Gómez-Reyes et al., 2018; Guevara et al., 2015; Piña-Páez et al., 2018).

Carya illinoinensis K. Koch (pecan) is an agronomically important ectomycorrhizal nut tree in whose plantations in the southern United States truffle species such as *Tuber lyonii* have been reported (Benucci et al., 2012; Bonito et al., 2011; Rodríguez et al., 2018). Ascomata and ectomycorrhizae of *Tuber brennemanii* and *T. floridanum* have also been found in these orchards (Grupe et al., 2018). In Mexico, *Tuber* diversity has not been explored in pecan plantations, although due to their particular soil and climatic conditions it is to be expected that there are species not yet described. In this paper, *Tuber caryophilum* sp. nov., a species in the Rufum clade, whose distribution includes Chihuahua and la Comarca Lagunera of Coahuila, Mexico, is described based on morphological, ecological and molecular characters.

Materials and methods

Ascomata were collected from a *Carya illinoinensis* orchard in the Comarca Lagunera of Coahuila and characterized following the recommendations of Castellano et al. (1989) and Pegler et al. (1993). Duplicates of the specimens were deposited in the José Castillo Tovar herbarium (ITCV) and MEXU. Characters examined included ascoma size and color, asci shape, asci wall thickness, and number of spores per ascus. Sections were cut by hand and then mounted in 5% KOH and Melzer's reagent for light microscopy. Thirty measurements of different structures such as spores and asci were made in 5% KOH. Microscopic structures were measured and photographed on a Velab VE-B3 microscope and a ZEISS Scope A1 optical stereoscope.

DNA was extracted by the CTAB method and amplified by PCR in 25 µl reactions according to Sambrook et al. (1989). The reactions consisted of 2.5 µl of 10X PCR buffer, 2.0 µl of 2.5 Mm MgCl final concentration, 2.0 µl of dNTPs, 2.0 µl of each primer 10 µM, 1.5 units of Taq polymerase (GoTaq®, Promega, WI), 11.3 µl of MiliQ grade water and 3 µl of DNA. The ribosomal internal transcribed spacer (ITS) region was amplified with the ITS4 and ITS5 oligonucleotides. The PCR program consisted of an initial denaturation at 94 °C for 3 min, followed by 34 cycles of 94 °C, 51 °C and 72 °C for 1 min each and a final extension at 72 °C for 8 min (Taylor et al., 2006). Amplification was carried out on a MiniAmp Plus Thermal Cycler (Applied Biosystems, USA). A section of the large ribosome subunit (LSU) was amplified with the LR0R and LR5 oligonucleotides (Vilgalys & Hester, 1990) and the enzyme Taq & Load (Avantor, PA, USA).

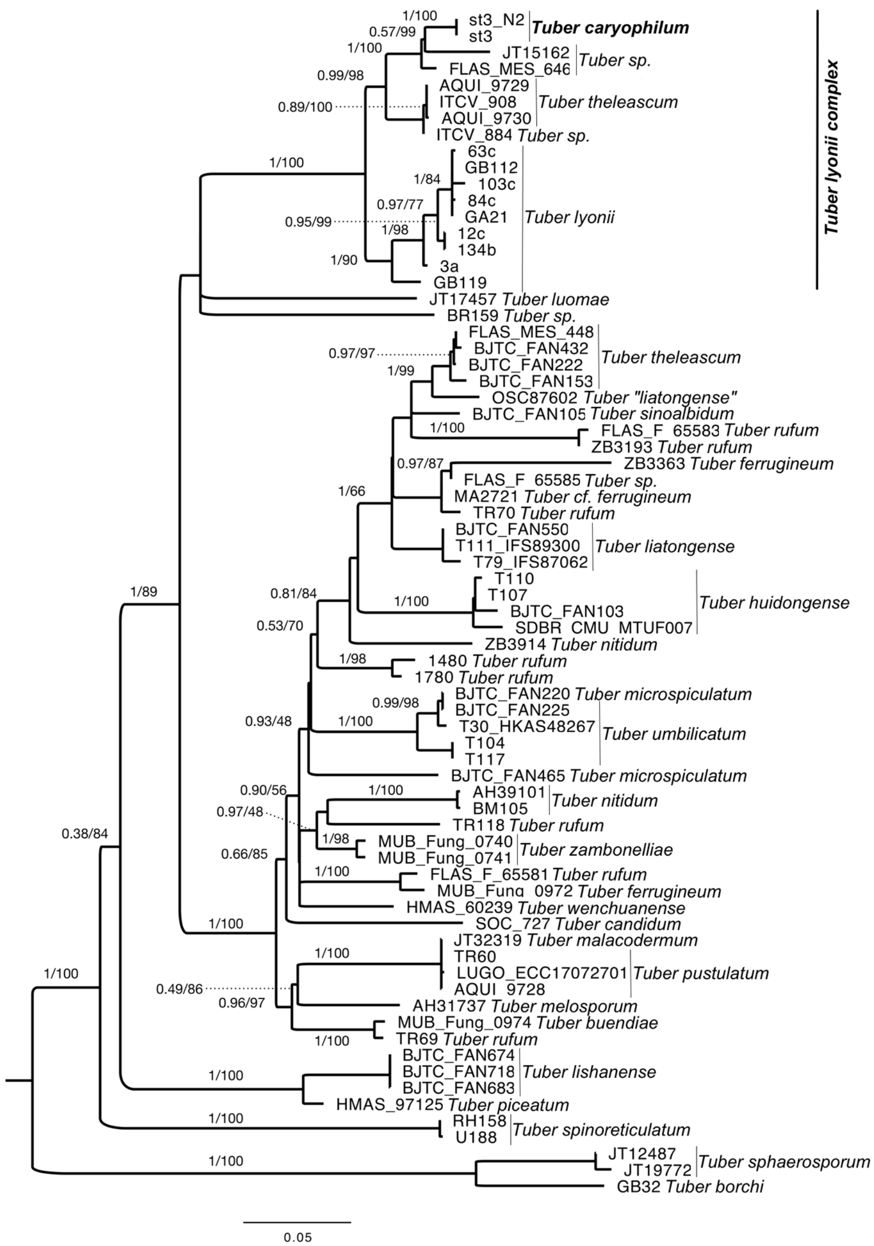

Figure 1. Phylogenetic placement of *Tuber caryophilum* sp. nov. in the Rufum clade. The tree is based on an ITS and LSU rDNA concatenated alignment. The consensus tree represents a Bayesian approximation with 1,000 generations and a maximum likelihood analysis with 1,000 bootstrap replicates. Just support values higher than 0.70 and/or 70% are displayed.

The 25 µl reactions consisted of 5 µl of master mix, 0.25 µl of each oligonucleotide 50 µM, water, and 1 µl of DNA. The PCR program began with denaturation at 94 ºC for 4 min, followed by 35 cycles at 94 ºC, 54 ºC and 72 ºC for 1 min each and final extension at 72 ºC for 10 min. PCR products were cleaned with ExoSAP-IT (Thermofisher, USA) with some modifications (Ángeles-Argáiz et al., 2016). DNA and PCR products were reviewed on 0.8% and 1.5% agarose gels with 0.5% TBE buffer. Samples were stained with Gel Red (Bioitium, CA, USA) using a 100 bp molecular weight marker as reference. Gels were photographed on a Multidoc-IT photodocumenter (Analytik

Table 1
Tuber species and DNA sequences used in the phylogenetic analyses. New sequences are in bold.

Voucher/Isolate	Species	ITS	28S	Country
GB32	*Tuber borchii*	FJ809799	FJ809799	Italy
MUB_Fung-0974	*Tuber buendiae*	MT006095	NG_073829	Spain
SOC_727	*Tuber candidum*	AY830856	-	
st3	***Tuber caryophilum***	**MZ092919**	**OK642388**	**Mexico**
st3_N2	***Tuber caryophilum***	**OK642397**	**OK642398**	**Mexico**
MA2721	*Tuber cf. ferrugineum*	-	FJ809809	
BJTC_FAN465	*Tuber crassitunicatum*	MH115295	-	
MUB_Fung-0972	*Tuber ferrugineum*	MN962719	-	Spain
ZB3363	*Tuber ferrugineum*	-	MT270600	Hungary
BJTC_FAN103	*Tuber huidongense*	MH115294	MH115301	China
SDBR-CMU-MTUF007	*Tuber huidongense*	KT758731	KU207733	Thailand
T107	*Tuber huidongense*	-	GU979099	China
T110	*Tuber huidongense*	FJ797882	GU979093	China
BJTC_FAN550	*Tuber liaotongense*	MH115302	-	China
OSC87602	*Tuber liaotongense*	-	FJ809813	China
T111_IFS89300	*Tuber liaotongense*	GU979037	-	
T79_IFS87062	*Tuber liaotongense*	GU979036	-	
BJTC_FAN674	*Tuber lishanense*	MH115307	-	China
BJTC_FAN683	*Tuber lishanense*	MH115305	-	China
BJTC_FAN718	*Tuber lishanense*	NR_160619	NG_064527	China
JT17457	*Tuber luomae*	MH142474	FJ809812	USA
103c	*Tuber lyonii*	GQ379726	GQ379726	USA
12c	*Tuber lyonii*	GQ379723	GQ379723	USA
134b	*Tuber lyonii*	GQ379724	GQ379724	USA
3a	*Tuber lyonii*	GQ379725	GQ379725	USA
63c	*Tuber lyonii*	GQ379722	GQ379722	USA
84c	*Tuber lyonii*	GQ379721	GQ379721	USA
GA21	*Tuber lyonii*	-	JQ925698	USA
GB112	*Tuber lyonii*	EU394704	EU394704	USA
GB119	*Tuber lyonii*	FJ748911	FJ809808	USA
JT32319	*Tuber malacodermum*	FJ809889	JQ925702	Spain
AH31737	*Tuber melosporum*	JN392144	JN392202	Spain
BJTC_FAN220	*Tuber microspiculatum*	MH115315	MH115316	China
AH39101	*Tuber nitidum*	JX402092	JN392331	
BM105	*Tuber nitidum*	FJ809885	FJ809807	Spain
ZB3914	*Tuber nitidum*	-	MT270604	Hungary
HMAS_97125	*Tuber piceatum*	NR_160620	NG_064528	China
AQUÍ_9728	*Tuber pustulatum*	-	MK211311	France
LUGO_ECC17072701	*Tuber pustulatum*	MW376716	-	Spain

Table 1. Continued

Voucher/Isolate	Species	ITS	28S	Country
TR60	*Tuber pustulatum*	MW077451	MW076943	
1480	*Tuber rufum*	EF362476	-	Italy
1780	*Tuber rufum*	EF362474	-	France
FLAS_F-65581	*Tuber rufum*	MT374048	-	France
FLAS_F-65581	*Tuber rufum*	-	MT350486	France
TR118	*Tuber rufum*	-	MT270605	Italy
TR69	*Tuber rufum*	-	MT270608	Spain
TR70	*Tuber rufum*	-	MT270602	Spain
ZB3193	*Tuber rufum*	-	MT270603	Slovakia
BJTC_FAN105	*Tuber sinoalbidum*	MH115298	MH115299	China
FLAS_MES-646	*Tuber sp.*	MT156470	-	USA
FLAS-F-65585	*Tuber sp.*	-	MT350482	France
JT15162	*Tuber sp.*	HM485391	-	USA
BR159	*Tuber sp. BR-2020a*	-	MW579345	USA
JT12487	*Tuber sphaerosporum*	FJ809853	FJ809853	USA
JT19772	*Tuber sphaerosporum*	FJ809854	FJ809854	USA
RH158	*Tuber spinoreticulatum*	GQ221454	FJ809814	USA
U188	*Tuber spinoreticulatum*	FJ809884	NG_059919	USA
BJTC_FAN153	*Tuber subglobosum*	-	MH115322	China
BJTC_FAN222	*Tuber subglobosum*	KF002728	MH115324	China
BJTC_FAN432	*Tuber subglobosum*	MH115323	-	China
FLAS_MES-448	*Tuber subglobosum*	MT156449	MT156449	China
AQUI_9729	*Tuber theleascum*	MK211283	MK211312	Mexico
AQUI_9730	*Tuber theleascum*	MK211284	MK211313	Mexico
ITCV_884	*Tuber theleascum*	HM485426	-	Mexico
ITCV_908	*Tuber theleascum*	NR_164592	-	Mexico
BJTC_FAN225	*Tuber umbilicatum*	MH115325	MH115326	China
T104	*Tuber umbilicatum*	FJ797879	-	
T117	*Tuber umbilicatum*	FJ797880	-	
T30_HKAS48267	*Tuber umbilicatum*	GU979032	GU979088	China
HMAS_60239	*Tuber wenchuanense*	JX267044	MH115327	Italy
MUB_Fung-0740	*Tuber zambonelliae*	MW632952	-	Spain
Mub_Fung-0741	*Tuber zambonelliae*	MW632953	-	Spain

Jena Company, CA, USA). ITS PCR products were sequenced in both directions at Macrogen (Rockville, MD, USA), with PCR primers. LSU sequences were obtained at the Biodiversity and Health Sequencing Laboratory of the Institute of Biology, UNAM, using BigDye Terminator 3.1 (Thermofisher), also in both directions.

Nucleotide sequences were edited and aligned in Geneious Prime version 2021 with the MUSCLE algorithm (Maddison & Maddison, 2016). Sequences of *T. caryophilum* voucher materials were deposited in GenBank under accession numbers MZ092919 and OK642388 for ITS, OK642397 and OK642398 for LSU and OK642406 for the ITS of ectomycorrhizae. The alignment for phylogenetic analyses included the sequences generated in this study, those previously included in analyses of the Rufum clade (Eberhart et al., 2020; Leonardi et al., 2019) and sequences of high nucleotide similarity obtained from the GenBank database by means of the BLAST algorithm (Altschul et al., 1990) (Table 1). A total of 72 samples from 26 taxa of the Rufum clade and 1 outgroup were aligned (Fig. 1). The concatenated alignment had 1,076 bp where bases 1-556 corresponded with the ITS and bases 557-1,076 with the LSU. The alignments were reviewed manually excluding ambiguous regions.

Phylogenetic analyses and evolutionary model selection were performed using IQ-TREE (v2.1.4, Minh et al., 2020) from the concatenated and partitioned alignment (IQ-TREE execution line: iqtree -s ../data/concat.fasta -p Partition.nex -m MFP --runs 100 --abayes -B 1000 -T AUTO -ntmax 28). The best evolutionary models were selected with ModelFinder (Kalyaanamoorthy et al., 2017). The best evolutionary model for the ITS marker was TIM2+F+I+G4 and for LSU it was TIM3e+I+G4. The resulting tree is the consensus of 100 replicates of 2 phylogenetic analyses; an ultra-fast Maximum Likelihood analysis (Hoang et al., 2018) with 1,000 bootstrap replicates (MVB), complemented with a Bayesian approximation branch support (BPP) analysis (Anisimova et al., 2011). To show the ectomycorrhizal status and distribution of *T. caryophilum*, the ITS sequence of the holotype was contrasted by means of nucleotide similarity (% ITS NS) against ectomycorrhizal sequences of *Carya illinoinensis* obtained from an orchard in Chihuahua, Mexico.

Results

The consensus tree of Bayesian approximation and Maximum likelihood shows the Rufum clade as monophyletic and with high support (BPP = 1, MV = 100). Within this clade, *T. caryophilum* is an independent, monophyletic and well-supported clade (BPP = 1, MVB = 100). This species appears as the sister clade of *T. theleascum* (BPP = 0.99, MVB = 98). In turn, these 2 species are grouped with the *T. lyonii* complex in a well-supported clade (BPP = 1, MVB = 100) made up of species from the southern USA and northern Mexico. Moreover, we found that the ITS sequence of the *T. caryophilum* holotype had a 99.3% NS (4 substitutions/549 bp) with mycorrhizal sequences from a pecan orchard in Chihuahua. This indicates that, like *T. lyonii*, *T. caryophilum* is an ectomycorrhizal symbiont of *C. illinoinensis*. Consequently, *Tuber caryophilum* is designated as a new species supported by ITS and LSU phylogenetic analyses of rnDNA, morphological characters, and ecology.

Description

Tuber caryophilum J.A. Sánchez, G. Guevara and R. Garibay-Orijel, sp. nov.
Fig. 2a-f
MycoBank 840581
GenBank MZ092919 (ITS), OK642397 (LSU)

Type. Mexico, Coahuila, Municipality of Viesca, Tierra Blanca Orchard, September 4, 2019, Sánchez st3 (ITCV 1888 "José Castillo Tovar" herbarium).

Diagnosis. Cream peridium with translucent veins towards the epicutis; pseudoparenchymatous epicutis mainly although in some areas it is prosenchymatous, isodiametric hyphae of 3-17 µm; echinulate ascospores of 18-48 × 10-27 µm; it grows in pecan (*Carya illinoinensis*) orchards.

Ascoma. Subglobose to irregular, 19 × 10 × 18 mm, translucent veins on the light brown to dark brown peridium when dry, with a white to cream furrow and an irregular linear or "V"-shaped margin continuing into the gleba as veins, some areas dark brown with cherry tints and with dark brown to reddish insect caverns; peridium smooth, some areas rough, separable from the gleba, without cystidia. White, cream gleba that is gray to dark when dried, marbled with white to gray, dark brown to reddish brown veins that continue towards the peridium (furrows). Strong, very pleasant and distinctive odor, unrecorded taste.

Peridium. 110-220 µm thick, epicutis 50-75 µm, pseudoparenchymatous in its outermost part, although in some parts it is prosenchymatous with hyphae 3-17 µm wide, versiform to angular or isodiametric, wall 1-4 µm thick, yellow to orange-reddish in KOH; subcutis 70-150 µm wide, pseudoparenchymatous strongly interwoven, hyaline hyphae in KOH, septate 2-4 µm wide. Gleba, intertwined vein hyphae, 2-4 µm at widest part. Asci: 47-105 × 32-50 µm (Q = 1.07-2.63), average 67.1 × 40.6 µm (Q = 1.7) including pedicel, subglobose to broadly ellipsoid, hyaline in KOH, 1-2 µm double wall may have a short to very long pedicel or in some it is absent, 1-5 ascospores per ascus. Ascospores: 18-48 × 10-27 µm (Q = 1-2.40), average 30.1 × 17.2 (Q = 1.76) subglobose to broadly ellipsoid or spindle-shaped excluding ornamentation; echinulate, echinulae mostly free, in some of them a subreticula can be observed, 1-4 µm high. Asci with 1 ascospore 35-48 × 18-27 µm (Q = 1.30-2.33), average 42.1 × 22.4 (Q = 1.91); 2 ascospores 20-38 × 15-21µm (Q = 1-2.4), average 31.3 × 17.7 µm (Q = 1.79); 3 ascospores 18-33 × 14-18 µm (Q = 1.06-2), average 27.1 × 16.6 (Q = 1.63); 4 ascospores 21-33 × 12-18 µm (Q = 1.44-2.13), average 26.4 × 15.1 (Q = 1.76); 5 ascospores 19-33 × 10-17 µm (Q = 1.19-2.36), average 24 × 14.4 (Q = 1.69).

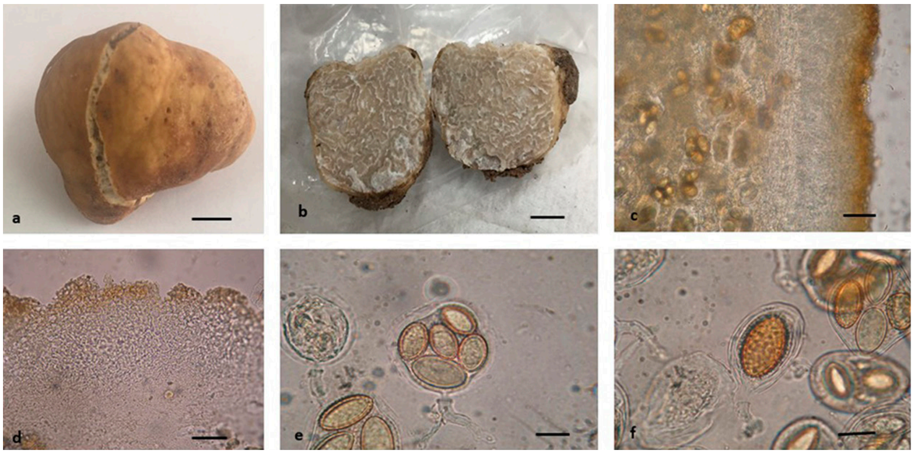

Figure 2. *Tuber caryophilum* (holotype ITCV 1888). a, Ascomata showing peridial surface; b, ascoma in cross section showing glebal surface; c, peridium cross section showing pseudoparenchyma; d, peridium in cross section; e, ascospores within asci showing alveoli; f, ascospore with equinulate surface. a, b (bar = 1 cm); c, d, e, f (bar = 20μm).

Taxonomic summary

Etymology. Refers to the ectomycorrhizal association between *T. caryophilum* and *Carya illinoinensis.*

Distribution and ecology. In northern Mexico in la Comarca Lagunera of Coahuila and Chihuahua, ectomycorrhizal symbiont of *Carya illinoinensis*. To date it has only been found in pecan orchards, not in natural habitats.

Habitat. Hypogeous, solitary or gregarious under pecan trees (*Carya illinoinensis*).

Collections examined in Mexico. Coahuila, Municipality of Viesca, Tierra Blanca Orchard, September 4, 2019, Sánchez st3_N2, MEXU 30227; Sánchez st3_N3, ITCV 1890; Sánchez st3_N4, ITCV 1891.

Remarks

Phylogenetic analyses show that *Tuber caryophilum* belongs to the Rufum clade and, together with *T. theleascum* (ITS NS = 93.6-93-9%), is related (ITS NS = 90.4-93.1%) to the *T. lyonii* complex*,* which is also an ectomycorrhizal species of *Carya illinoinensis*. *Tuber caryophilum* differs from *T. theleascum* because the latter has ascomata without translucent areas on the peridium, a pseudoparenchymatous epicutis with elongated prostrate or intertwined hyphae 4-7 μm wide and smooth, while the former species has translucent areas on the peridium and a pseudoparenchymatous epicutis with isodiametric hyphae 13-17 μm wide. Furthermore, they differ in ascospores size; *T. caryophilum* has 18-48 × 10-27 μm ascospores, whereas in *T. theleascum* they are 18-44 × 13-25 μm. *Tuber caryophilum* is also similar to *T. lyonii,* an edible truffle species native to the southeastern USA (Bonito et al., 2013; Sharma et al., 2012). However, they differ macro- and microscopically; in *T. lyonii*, peridium width is larger (300-500 μm), ascospores are ellipsoid 30-37 × 22-24 μm and epicutis width is 20-40 μm, with hyphae 6-10 μm wide (Healy et al., 2016; Sharma et al., 2012). In contrast, in *T. caryophilum,* the peridium width is 110-220 μm, its ascospores are 18-48 × 10-27 μm and epicutis width is 50-75 μm, with 3-17 μm hyphae. These 3 species share important microscopic features such as the pseudoparenchymatous peridium surface and most of their ascospores are subglobose to ellipsoid. Other common features are that all 3 are closely related in the same clade of the Rufum section and that they develop in pecan orchards (Grupe et al., 2018; Sharma et al., 2012; Trappe et al., 1996).

Other *Tube*r species associated with pecan plantations have been described but these do not belong to the Rufum section (Table 2). One of these species is *Tuber brennemanii*, which belongs to the Maculatum clade and therefore differs morphologically and molecularly from species belonging to the Rufum clade. For example, *T. brennemanii* presents anamorphic ascospores and a periclinal subperidium (Grupe et al., 2018). Likewise, *Tuber floridanum*, also on the Maculatum clade, has been found in pecan orchards. This species is distinguished by the presence of dermatocystidia and commonly has 2-4 spores and reticulate ornamentation as present in the Maculatum (Grupe et al., 2018). Regarding *T. floridanum*, it is known to have been unintentionally introduced into southern Brazil on the roots of pecan tree seedlings.

Given the phylogenetic closeness of *T. caryophilum* to *T. lyonii*, its discovery in pecan orchards opens the door

Table 2
Tuber species related to *Tuber caryophilum* or associated with *Carya illinoinensis*.

Species	Peridium Surface	Peridium color and thickness	Epicutis / subcutis and cell size	Ascospores size without spines/ alv.	Ascospores shape	Ascopores by asci	Geography and host
Tuber caryophilum	Smooth, separable without dermatosictidia	Yellow to reddish orange 110-220µm	Ps. 50-75 µm, 3-17 µm / Pr. 70-150µm	18-48 × 10-27 µm	Subglobose to broadly ellipsoid	1-5	Comarca Lagunera, Mexico. *Carya illinoinensis*
Tuber lyonii	Smooth and slightly pruinose	Yellowish brown 300-500 µm	Ps. 20-40 µm, 6-10 µm	30-37 × 22-24 µm	Ellipsoid	1-4	Northeastern Mexico; Florida, USA. *Quercus*, *C. illinoinensis*
Tuber theleascum	Smooth	Yellow to reddish brown 160-250 µm	Ps. 45-150 µm, 4-7 µm / Pr. 150 µm	18-44 × 13-25 µm	Claviform to subglobose	1-4	Nuevo Leon, Mexico. *Quercus canbyi*, *Q. polymorpha*, *Q. laeta*, *Arbutus*
Tuber brennemanii	Smooth	Yellow to reddish brown 80-600 µm	Ps. 50-200 µm, 2.5-25 µm	28-61 × 20-36 µm	Isodiametric globose to subglobose	1-4	Nuevo Leon, Mexico; Massachusetts and Georgia, USA. *C. illinoinensis*, *Quercus*, and other Fagales
Tuber floridanum	Smooth	Reddish brown 300-1120 µm	Ps. 140-800 µm, 5-35 µm	36-51 × 26-38 µm	Isodiametric globose to subglobose	2-4	Florida, Georgia, and Mississippi, USA. *C. illinoinensis* and other Fagales

Ps: Pseudoparenchymatous; Pr: prosenchymatous.

to its use in northern Mexico. To develop this, it will be necessary to carry out mycorrhization experiments in nurseries and to know its organoleptic properties. This could promote a system of co-cultivation between *T. caryophilum* and *C. illinoinensis* as occurs in orchards in southeastern Florida and Europe where various species of truffles are harvested alongside nut production (Bonito et al., 2013; Lefevre et al., 2012; Trappe et al., 1996).

Acknowledgements

To Conacyt for the financial support and the producers of pecan for the access to the *Carya illinoinensis* orchards. GG, thanks TecNM for research support.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*, 403–410.

Anisimova, M., Gil, M., Dufayard, J. F., Dessimoz, C., & Gascuel, O. (2011). Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. *Systematic Biology*, *60*, 685–699. https://doi.org/10.1093/sysbio/syr041

Benucci, G. M. N., Bonito, G., Falini, L. B., & Bencivenga, M. (2012). Mycorrhization of Pecan trees (*Carya illinoinensis*) with commercial truffle species: *Tuber aestivum* Vittad. and *Tuber borchii* Vittad. *Mycorrhiza*, *22*, 383–392. https://doi.org/10.1007/s00572-011-0413-z

Benucci, G. M. N., Csorbai, A. G., Falini, L. B., Bencivenga, M., Di Massimo, G., & Donnini, D. (2012). Mycorrhization of *Quercus robur* L., *Quercus cerris* L. and *Corylus avellana* L. seedlings with *Tuber macrosporum* Vittad. *Mycorrhiza*, *22*, 639–646. https://doi.org/10.1007/s00572-012-0441-3

Bonito, G., Smith, M. E., Nowak, M., Healy, R. A., Guevara, G., Cázares, E. et al. (2013). Historical biogeography and diversification of truffles in the Tuberaceae and their newly identified southern hemisphere sister lineage. *Plos One*, *8*, e52765. https://doi.org/10.1371/journal.pone.0052765

Bonito, G., Brenneman, T., & Vilgalys, R. (2011). Ectomycorrhizal fungal diversity in orchards of cultivated pecan (*Carya illinoinensis*; Juglandaceae). *Mycorrhiza*, *21*, 601–612. https://doi.org/10.1007/s00572-011-0368-0

Castellano, M. A. (1989). *Key to spores of the genera of hypogeous fungi of north temperate forests with special reference to animal mycophagy*. Eureka, USA: Mad River Press.

Eberhart, J., Trappe, J., Páez, C. P., & Bonito, G. (2020). *Tuber luomae*, a new spiny-spored truffle species from the Pacific Northwest, USA. *Fungal Systematics and Evolution*, *6*, 299. https://doi.org/10.3114/fuse.2020.06.15

Fan, L., Han, L., Zhang, P. R., & Yan, X. Y. (2016). Molecular analysis of Chinese truffles resembling *Tuber californicum* in morphology reveals a rich pattern of species diversity with emphasis on four new species. *Mycologia*, *108*, 344–353. https://doi.org/10.3852/14-343

García-Jiménez, J., Ayala-Vásquez, O., Guevara-Guerrero, G., Garza-Ocanas, F., & De La Fuente, J. I. (2021). *Tuber mixtecorum* (Tuberaceae, Pezizales) a new truffle in the Maculatum clade from Mexico. *Phytotaxa*, *509*, 113-120.

Gómez-Reyes, V. M., Vázquez-Marrufo, G., Ortega Gómez, A. M., & Guevara Guerrero, G. (2018). Ascomicetos hipogeos de la región occidental del Sistema Volcánico Transversal, México. *Acta Botanica Mexicana*, *125*, 37–48. https://doi.org/10.21829/abm125.2018.1327

Grupe, A. C., Sulzbacher, M. A., Grebenc, T., Healy, R., Bonito, G., & Smith, M. E. (2018). *Tuber brennemanii* and *Tuber floridanum*: Two new *Tuber* species are among the most commonly detected ectomycorrhizal taxa within commercial pecan (*Carya illinoinensis*) orchards. *Mycologia*, *110*, 780–790. https://doi.org/10.1080/00275514.2018.1490121

Guevara, G., Bonito, G., & Cázares, E. (2013). Revisión del género *Tuber* (Tuberaceae: Pezizales) de México. *Revista Mexicana de Biodiversidad*, *84*, S39–S49. https://doi.org/10.7550/rmb.31981

Guevara, G., Bonito, G., Cázares, E., Rodríguez, J., Vilgalys, R., & Trappe, J. M. (2008). *Tuber regimontanum*, new species of truffle from Mexico. *Revista Mexicana de Micología*, *26*, 17–20.

Guevara, G., Bonito, G., Trappe, J. M., Cázares, E., Williams, G., Healy, R. A. et al. (2013). New North American truffles (*Tuber* spp.) and their ectomycorrhizal associations. *Mycologia*, *105*, 194–209. https://doi.org/10.3852/12-087

Guevara-Guerrero, G., Bonito, G., Cázares-González, E., Healy, R., Vilgalys, R., & Trappe, J. (2015). Novel *Tuber* spp. (Tuberaceae, Pezizales) in the *Puberulum* Group from Mexico. *Ascomycete.org*, *7*, 367–374. https://doi.org/10.25664/art-0161

Healy, R., Bonito, G. M., & Smith, M. E. (2016). A brief overview of the systematics, taxonomy, and ecology of the *Tuber rufum* clade. In A. Zambonelli, M. Iotti, & C. Murat, (Eds.), *True truffle* (Tuber spp.) *in the World* (pp. 125–136). Berlin: Springer. https://doi.org/10.1007/978-3-319-31436-5_8

Hoang, D. T., Chernomor, O., Von Haeseler A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, *35*, 518–522. https://doi.org/10.1093/molbev/msx281

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K., Von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, *14*, 587–589.

Kirk, P. M., Cannon, P. F., Minter, D. W., & Stalpers, J. A. (2008). *Ainsworth and Bisby's dictionary of the fungi*. Wallingford: CABI.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549. https://doi.org/10.1093/molbev/msy096

Lancellotti, E., Iotti, M., Zambonelli, A., & Franceschini, A. (2016). The Puberulum group sensu lato (whitish truffles). In A. Zambonelli, M. Iotti, & C. Murat (Eds.), *True truffle (*Tuber *spp.) in the World* (pp. 105–124). Berlin: Springer. https://doi.org/10.1007/978-3-319-31436-5_7

Lefevre, C. (2012). Native and cultivated truffles of North America. In A. Zambonelli, & G. Bonito (Eds.), *Edible ectomycorrhizal mushrooms* (pp. 209–226). Berlin: Springer. https://doi.org/10.1007/978-3-642-33823-6_12

Leonardi, M., Paz-Conde, A., Guevara, G., Salvi, D., & Pacioni, G. (2019). Two new species of *Tuber* previously reported as *Tuber malacodermum*. *Mycologia*, *111*, 676–689. https://doi.org/10.1080/00275514.2019.1603777

Li, Q., Yan, L., Ye, L., Zhou, J., Zhang, B., Peng, W. et al. (2018). Chinese black truffle (*Tuber indicum*) alters the ectomycorrhizosphere and endoectomycosphere microbiome and metabolic profiles of the host tree *Quercus* aliena. *Frontiers Microbiology*, *9*, 2202. https://doi.org/10.3389/fmicb.2018.02202

Maddison, W. P, & Maddison, D. R. (2016). Mesquite: a modular system for evolutionary analysis (Version 3.10) http://mesquiteproject.org

Marozzi, G., Sánchez, S., Benucci, G. M. N., Bonito, G., Falini, L. B., Albertini, E., & Donnini, D. (2017). Mycorrhization of pecan (*Carya illinoinensis*) with black truffles: *Tuber melanosporum* and *Tuber brumale*. *Mycorrhiza*, *27*, 303–309. https://doi.org/10.1007/s00572-016-0743-y

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, *37*, 1530–1534. https://doi.org/10.1093/molbev/msaa015

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford: Oxford University Press.

Neri-Luna, C., & Villarreal-Ruiz, L. (2012). Simbiosis micorrícica: un análisis de su relevante función ecosistémica y en la provisión de servicios ambientales. In M. Huerta, & F. Castro (Comps.), *Interacciones ecológicas* (pp. 37–61). Guadalajara: Universidad de Guadalajara. https://doi.org/10.1007/s00572-016-0743-y

Pegler, D. N., Spooner, B. M., & Young, T. W. K. (1993). *British truffles, a revision of British hypogeous fungi*. Kew, UK: Royal Botanic Gardens.

Pina-Paez, C., Bonito, G., Guevara-Guerrero, G., Castellano, M. A., Garibay Orijel, R., Trappe, J. M. et al. (2018). Description and distribution of *Tuber incognitum* sp. nov. and *Tuber anniae* in the Transmexican Volcanic Belt, *Mycokeys*, *41*, 17–27. https://doi.org/10.3897/mycokeys.41.28130

Rincón, A., Alvarez, I. F., & Pera, J. (2001). Inoculation of containerized *Pinus pinea* L. seedlings with seven ectomycorrhizal fungi. *Mycorrhiza*, *11*, 265–271. https://doi.org/10.1007/s005720100127

Rodríguez, R. A., Valdés, M. P., & Ortiz, S. (2018). Características agronómicas y calidad nutricional de los frutos y semillas de zapallo *Cucurbita* sp. *Revista Colombiana de Ciencia Animal Recia*, *10*, 86–97. https://doi.org/10.24188/recia.v10.n1.2018.636

Sambrook, J., Fritsch, E., & Maniatis, T. (1989). *Molecular cloning*: a *laboratory manual*. (No. Ed. 2). Long Cold Island, NY: Cold Spring Harbor Laboratory Press.

Sharma, J., Trela, B., Wang, S., Smith, M., & Bonito, G. (2012). Pecan truffle (*Tuber lyonii*) in Texas. *Pecan South*, *2012*, 16–24.

Trappe, J. M. (1979). The orders, families, and genera of hypogeous Ascomycotina (truffles and their relatives). *Mycotaxon*, *9*, 297–340.

Trappe, J. M., Jumpponen, A. M. J., & Cazares, E. (1996). NATS truffle and truffle-like fungi. 5. *Tuber lyonii* (= *T. texense*), with a key to the spiny-spored *Tuber* species groups. *Mycotaxon*, *60*, 365–372.

Vilgalys, R., & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, *172*, 4238–4246.