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# *Tuber itzcuinzapotl sp. nov.* (Tuberaceae, Pezizomycetes), the first edible truffle reported from Mexico with traditional biocultural importance

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## Abstract

*Tuber itzcuinzapotl* is described as a new species to science. This species is characterized by its pale brown ascomata, finely granular peridium, pale brown to gray gleba, and 22–52 × 15–40 µm alveolate ascospores. The new species is putatively associated with *Pinus patula* in conifer mixed forests in the state of Veracruz, located in eastern Mexico. Phylogenetic analysis based on the nrITS region places the new species in the Maculatum clade, closely related, but morphologically distinctive, to *T. miquihuanense* and *T. mexiusanum*. The new species consumed by the Nahua people, traditionally named "Itzcuinzapotl", constitutes the first edible truffle with biocultural importance in Mexico. Macro- and micromorphological characterization, results of phylogenetic analysis, and photographs are presented. Ethnomycological aspects related to the species are also briefly discussed.

**Key words:** 1 new species, biocultural erosion, ethnomycology, hypogeous fungi, Mexican truffles, mycorrhizal fungi, traditional knowledge

## Introduction

*Tuber* P. Micheli ex F.H. Wigg is a genus of the order Pezizales whose species, commonly known as truffles, are characterized by their hypogeous habits with echinulate or alveolate ascospores, and by presenting characteristic aromas that allow them to be dispersed by some invertebrates and vertebrates (Montecci & Sarasini 2000; Trappe *et al*. 2009). The genus *Tuber* includes ectomycorrhizal fungi associated with gymnosperms and angiosperms, mainly *Carya*, *Pinus*, *Quercus*, and even some orchid species. Ecologically, they play a role of paramount relevance in the nutrient cycling in forests (Bonito *et al.* 2012; Castellano *et al*. 1989; Guevara-Guerrero *et al*. 2013a). Currently, more than 230 species grouped into 11 clades around the world are known (Bonito *et al*. 2013). Due to their peculiar aroma and flavor, some species are highly appreciated in gourmet gastronomy and are traded internationally at annual-in season retail market higher than USD 150 million (Payen *et al*. 2014; Pérez-Moreno *et al*. 2021b). *Tuber* species can be either collected from natural forests or from artificial plantations e.g., *T. melanosporum* Vittad. is known as the Périgord black truffle (Guevara-Guerrero *et al*. 2022).

Currently, in Mexico, 25 species belonging to the genus *Tuber* have been recorded, mainly from northeastern temperate forests and the Neovolcanic axis, most of them without commercial value (Guevara-Guerrero *et al*. 2013a; García-Jiménez *et al*. 2021). Despite the fact that Mexico constitutes the second biocultural center of edible wild

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edible fungi in the world, only behind China (Pérez-Moreno *et al.* 2021b), in the country there is no commercial trade of *Tuber* species. However, recently the synthesis of the black truffle (*T. melanosporum*) with native oaks has been successfully achieved, which opens the possibility of developing an industry with high economic relevance (Guevara-Guerrero *et al*. 2022). Additionally, some species that grow in natural forests have potential economic importance, e.g., *T. regiomontanum* G. Guevara, Bonito & Julio Rodr., *T. canaliculatum* Gilkey, *T. bonitoi* G. Guevara & Trappe and *T. lyonii* Butters. This latter species, reported from northeastern Mexico (Guevara-Guerrero *et al.* 2013a), has an international price of up to USD 400 per kilogram (Healy *et al*. 2016).

Mexico is one of the diversification centers of the *Quercus* and *Pinus* genera, which have been reported to establish ectomycorrhizal symbiosis with species of the genus *Tuber*, and therefore the potential number of truffle species may be higher than those reported so far (García-Jiménez 2014). In the present study, a new edible *Tuber* species, grew under *Pinus patula* Schiede ex Schltdl. & Cham trees in coniferous mixed forests in eastern Mexico, with traditional biocultural importance among Nahua people, is described. Morphological characterization, photographs, and phylogenetic analysis based on the ITS region are presented.

## Material and methods

### *Collection site and sample collection*

The study area is the Mexcala locality, municipality of Soledad Atzompa, Veracruz state located in eastern Mexico. The area is dominated by *Pinus patula* plantations, and natural forests of *P. patula*, *P. ayacahuite* Ehrenb. Ex Schltdl., *Abies hickelli* Flous & Gaussen, *Arbutus xalapensis* Kunth and *Cupressus* spp. The collection of the specimens was carried out following the protocols of Castellano *et al*. (1989) in the company of local people, two of them being coauthors of this contribution. The collected specimens were photographed and dried at 40°C (Hu *et al.* 2022). Temporal slides were made using 5% KOH and Melzer's reagent to observe and measure the peridium hyphae, asci, and ascospores. The reviewed material is deposited in the herbarium of the Instituto Tecnológico Superior de Zongolica (ZON), Veracruz state.

### *Extraction, amplification, and sequencing of DNA*

Genomic DNA was obtained with the CTAB method (Martínez-González *et al*. 2017) using 2-3 mg of dried glebal tissue. The PCR amplification included 35 cycles with an annealing temperature of 54 ºC and was carried out with the ITS5 and ITS4 primers (White *et al*. 1990) for the ITS nrDNA region. The PCR products were verified by agarose gel electrophoresis. The gels were run for 1 h at 95 V $cm^{-3}$ in 1.5% agarose and 1× TAE buffer (Tris Acetate-EDTA). The gel was stained with GelRed (Biotium, USA) and the bands were visualized in an Infinity 3000 transilluminator (Vilber Lourmat, 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 Biosystems model 3730XL (Applied BioSystems, USA), at the Instituto de Biología of the Universidad Nacional Autónoma de México (UNAM).

### *Sequence assembly*

The sequences of both strands of each of the genes were analyzed, edited, and assembled using BioEdit v.7.0.1 (Hall 2004) to generate a consensus sequence. These consensus sequences were compared with those deposited in the GenBank of the National Center for Biotechnology Information (NCBI), using the tool BLAST 2.2.19 (Zhang *et al*. 2000).

### *Phylogenetic analyses*

To study phylogenetic relationships, our newly produced sequences of two individuals of the studied species were added to reference sequences of nrITS deposited in the NCBI database (http://www.ncbi.nlm.nih.gov/genbank/). The nrITS dataset included sequences from 54 specimens representing 36 taxa. The nrITS region was aligned using the online version of MAFFT v. 7 (Katoh *et al*. 2002; Katoh & Standley 2013). The alignment was revised 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 54 individuals (685 characters). The data were analyzed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). Maximum parsimony analyses were carried out in PAUP* 4.0b10 (Swofford 2002) using the heuristic search mode, 1000 random starting replicates, and TBR branch swapping, with MULTREES and Collapse on. Bootstrap values were estimated using 1000 bootstrap replicates under the heuristic search mode, each with 100 random starting replicates. Maximum likelihood analyses were carried out in IQ-tree ver. 1.6.12 (Nguyen *et al*. 2015) with a GTR + G model of nucleotide substitution. To assess Branch support, 1000 rapid bootstrap replicates were run with the GTRGAMMA model. Bayesian inference was carried out in MrBayes v.3.2.7 x64 (Ronquist *et al*. 2012) with four chains and the best evolutionary model for alignment was sought using Partition Finder (Lanfear *et al.* 2014; 2017), the best-fit model selected for these three partitions of nrITS sequences was GTR+G for ITS1, JC for 5.8s, and HKY+G for ITS2. The information block for the matrix includes 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 convergence was evaluated by examining the standard deviation of Split frequencies among runs and by plotting the log-likelihood values from each run using TRACER v.1.7.1 (Rambaut *et al.* 2018). The remaining trees were used to calculate a 50% majority-rule consensus topology and posterior probabilities (PP). Trees were visualized and optimized in FigTree v.1.4.4 (Rambaut 2018).

## Results

### *Molecular analyses*

We successfully amplified and sequenced the nrITS region from two specimens of the studied species. After incorporation of additional sequences downloaded from GenBank (Table 1), the aligned nrITS dataset included 706 characters (including gaps), of which 264 conserved sites, 435 variable sites, and 349 parsimony informative sites. The three phylogenetic analyses, MP, ML, and BI, of the nrITS dataset recovered similar topologies (Fig. 1). No significant conflict (bootstrap value70%) was detected among the topologies obtained via the separate phylogenetic analyses. The parsimony analysis of the alignment found 851 trees of 245 steps (CI=0.2541, HI=0.1041, RI=0.4485, RC=0.6877). The best RAxML tree with a final likelihood value of –40578.924927 is presented. The matrix had 1001 distinct alignment patterns, with 5.65% undetermined characters or gaps. Estimated base frequencies were as follows: A= 0.102548, C= 0.207510, G= 0.104213, T= 0.296420; substitution rates AC= 1.080347, AG= 1.507910, AT= 1.151281, CG= 1.008021, CT= 5.407841, GT= 1.000000; gamma distribution shape parameter α= 0.003014. In the Bayesian analysis, the standard deviation between the chains stabilized at 0.00002 after 4 million generations. No significant changes in tree topology trace or cumulative Split frequencies of selected nodes were observed after about 0.25 million generations, which were discarded as 25% burn-in. In the present study, a new species based on morphological characters and phylogenetic analysis of nrITS (GenBank accession number OR429351- OR429352) sequences is described (Fig. 1). The analysis of nrITS produced a phylogenetic tree where *Tuber itzcuinzapotl* is shown as a monophyletic group (BS = 100%, BS = 100%, BI p = 1), related to *T. mexiusanum* Guevara, Bonito & Cázares and *T. miquihuanense* G. Guevara, Bonito & Cázares.

**TABLE 1.** Species, voucher numbers, countries and corresponding GenBank accessions of the *Tuber* specimens used for the phylogenetic analysis.

| Species | Voucher | Country | ITS |
|---|---|---|---|
| *Tuber alboumbilicum* | YAAS L2324 | China | KJ742702 |
| *Tuber asa* | M1828 | Italy | HM485341 |
| *Tuber aztecorum* | GG1109 | Mexico | KY271790 |
| *Tuber aztecorum* | GG993 | Mexico | KY271791 |
| *Tuber beyerlei* | OSC 130875 | USA | NG042577 |
| *Tuber bomiense* | SKM106 | China | KC517481 |

*......continued on the next page*

**TABLE 1.** (Continued)

| Species | Voucher | Country | ITS |
|---|---|---|---|
| *Tuber bonitoi* | JT32421 | Mexico | KT897472 |
| *Tuber bonitoi* | ITCV913a | Mexico | KT897473 |
| *Tuber borchii* | GB62 | Italy | HM485342 |
| *Tuber brunneum* | JT33835 | Mexico | KT897474 |
| *Tuber brunneum* | JT33836 | Mexico | KT897475 |
| *Tuber brunneum* | JT33830 | Mexico | KT897478 |
| *Tuber californicum* | JT28058 | USA | HM485346 |
| *Tuber castilloi* | ITCV 149 | Mexico | NR119865 |
| *Tuber cistophilum* | AH39275 | Spain | JN392231 |
| *Tuber dryophilum* | GB37 | Italy | HM485354 |
| *Tuber foetidum* | ZB516 | Hungary | JQ288907 |
| *Tuber huizeanum* | FAN186 | China | JQ910651 |
| ***Tuber itzcuinzapotl*** | **52-ZON Holotype** | **Mexico** | **OR429351** |
| ***Tuber itzcuinzapotl*** | **53 ZON** | **Mexico** | **OR429352** |
| *Tuber latisporum* | HKAS 44315 | China | NR119620 |
| *Tuber lauryi* | OSC 130885 | USA | MT156528 |
| *Tuber linsdalei* | L63 | USA | HM485370 |
| *Tuber lijiangense* | HKAS52005 | China | KF805727 |
| *Tuber maculatum* | FLAS: MES-885 | USA | MT156493 |
| *Tuber maculatum* | GO-2008-144 | Mexico | KJ595014 |
| *Tuber maculatum* | Db-A | Poland | MH040280 |
| *Tuber mixtecorum* | 21880 ITCV | Mexico | MW209737 |
| *Tuber mexiusanum* | ITCV 3785 | Mexico | HM485412 |
| *Tuber mexiusanum* | ITCV 181 | Mexico | HM485411 |
| *Tuber microsphaerosporum* | BJTCFan152 | China | KF805726 |
| *Tuber microverrucosum* | LY-2012a | China | JN870099 |
| *Tuber miquihuanense* | ITCV 885 | Mexico | HM485414 |
| *Tuber panzhihuanense* | DXJ260 | China | JQ978644 |
| *Tuber pseudomagnatum* | BJTC FAN 403 | China | OM265246 |
| *Tuber pseudomagnatum* | BJTC FAN 532 | China | OM265248 |
| *Tuber pseudomagnatum* | BJTC FAN 391 | China | OM365244 |
| *Tuber pseudoseparans* | JT33778 | Mexico | KT897480 |
| *Tuber pseudoseparans* | JT33774 | Mexico | KT897481 |
| *Tuber pseudosphaerosporum* | BJTCFan250 | China | KF744063 |
| *Tuber rapaeodorum* | CMI-UNIBO 2483 | Italy | DQ011849 |
| *Tuber separans* | JT7697 | USA | HM485385 |
| *Tuber shearii* | OSC51052 | USA | HM485389 |
| *Tuber sinosphaerosporum* | BJTC: FAN135 | China | JX092086 |
| *Tuber* sp. | 14 KA-2010 | Japan | AB553464 |
| *Tuber* sp. | 14 GB-2010 | Mexico | GQ221447 |
| *Tuber* sp. | JT8755 | USA | HM485416 |
| *Tuber* sp. | 48D | Mexico | JF419253 |
| *Tuber* sp. | RH974 | USA | JF419256 |
| *Tuber tequilanum* | JT33796 | Mexico | KT897482 |
| *Tuber tequilanum* | JT33755 | Mexico | KT897485 |

*......continued on the next page*

**TABLE 1.** (Continued)

| Species | Voucher | Country | ITS |
|---|---|---|---|
| *Tuber tequilanum* | JT33790 | Mexico | KT897486 |
| *Tuber vesicoperidium* | I155 | China | JQ690071 |
| *Tuber zhongdianense* | wang0299 | China | DQ898187 |

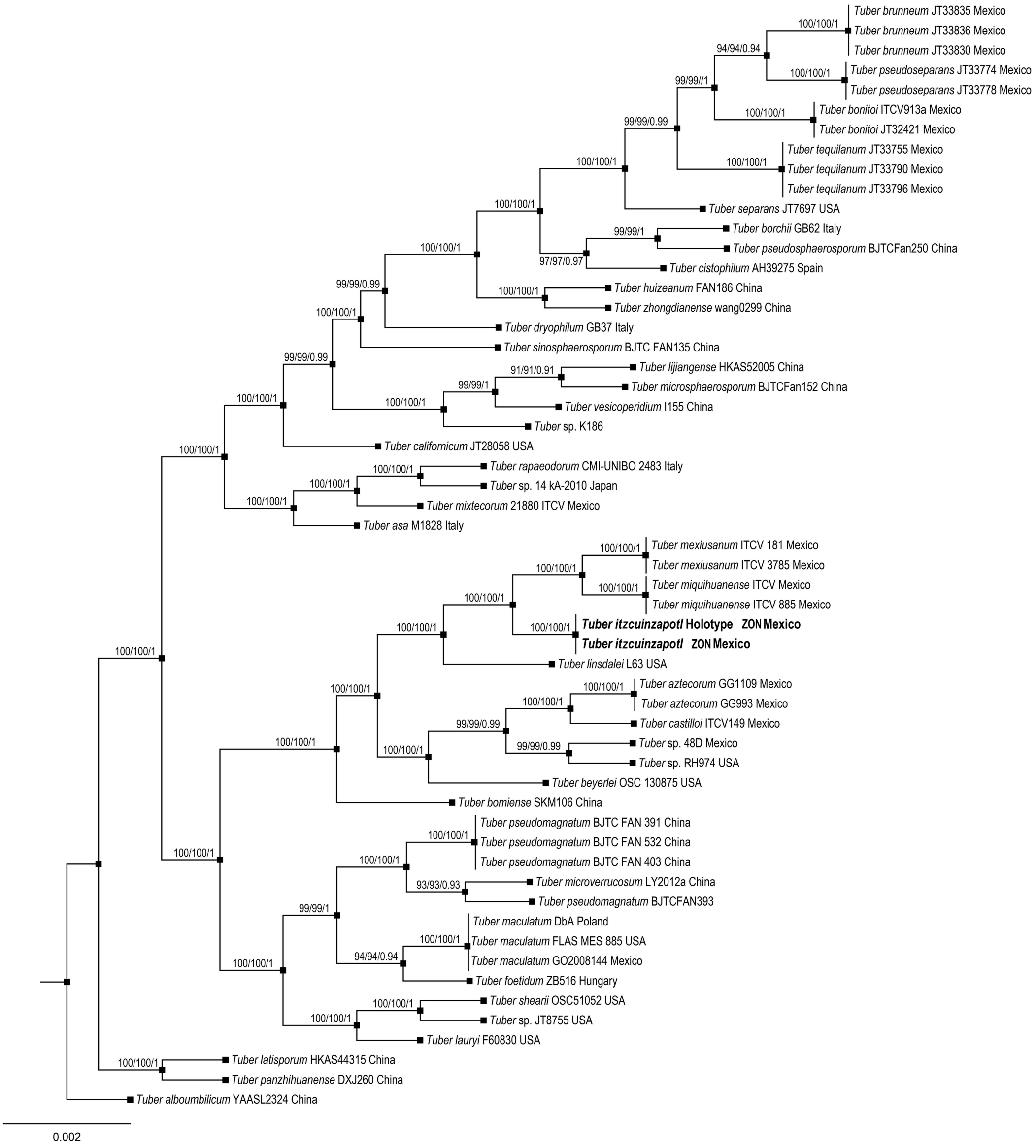

**FIGURE 1**. Maximum likelihood phylogeny based on the nrITS sequences data. Maximum parsimony and Bayesian analyses recovered identical topologies with respect to the relationships among the main clades of the *Tuber*. For each node, the following values are provided: maximum parsimony bootstrap (%) /maximum likelihood bootstrap (%) / and posterior confidence (p-value). The scale bar represents the expected number of nucleotide substitutions per site. The new species *Tuber itzcuinzapotl* is shown in bold. Sequences obtained from this study are in bold.

## Taxonomy

### ***Tuber itzcuinzapotl*** **de la Fuente & Rosales-Rosales, *sp. nov.***

Mycobank number: MB 84987, GenBank: OR429351 and OR429351

**Etymology:**—Refers to the name used by the Nahua people to designate this edibles species *itzcuinzapotl* (*itzcuin*=dog, and *zapotl*=zapote, a native sweet fruit).

*Ascomata* 35 × 28 mm, subglobose irregular to lobate, pale brown, darkening when touched, finely verrucose or granulose, 5−7 verrucae per mm, dry texture, without rhizomorphs at the base. *Peridium* thinner than 1 mm in width, grey, brown, yellowish when dry. *Gleba* marbled, pale brown to greyish brown, dark brown when mature, with abundant white veins, some coalescing in the peridium. *Taste* and *smell* fruity. *Peridium* 200−400 µm composed of two layers: *Epicutis* 180−250 µm, composed of a pseudparenchymatous layer composed of hyaline, inamyloid, thin-walled, 5−20 µm subglobose to globose hyphae in diameter, with thick, brownish cell-walls, usually forming pyramid-like structures of 150−200 × 60−180 µm, with scarce erect clavate hyphae of 11−30 × 6−10. *Subcutis* 80-150 µm width, composed of strongly interwoven hyphae, prosenchymatous in some areas, 4−12 µm in diameter, tubulose, rarely subglobose, and occasionally inflated near the septa, hyaline, inamyloid, thin-walled. *Veins* composed of strongly interwoven hyphae, 2−16 µm in diameter, hyaline, tubulose to inflate near the septa, hyaline, thin-walled, like those from the subcutis. *Asci* 34−70 × 20−50 µm, globose to subglobose, hyaline, some with a globose pedicel reaching up to 10 µm, mostly 2-spored, monosporic, rarely 3 or 4-spored, thick-walled (4 µm in diameter). *Ascospores* ellipsoid, pale brown to light brown, with angular alveoli of (2)5−10 × 3−5(7) µm, 5−8 sides, projecting up to 6 µm, thick-walled (up to 3 µm); 1-spored asci have ascospores of 40−52 × 23−30 µm; 2-spored asci have ascospores of 27−34 × 15−22 µm, 3-spored asci have ascospores of 22−39 × 15−19 and 4-spored asci have ascospores of 25−42 × 13−30 µm.

**Holotype:**—MEXICO. Veracruz: Soledad Atzompa municipality, Mexcala town, 18° 41' 46" N, 97° 10' 00" W, 2400 m, 17 April 2023, Wendy Rosales-Rosales, (52-ZON, holotype designated here).

**Habitat, habit, distribution:**—Solitary. So far, it is only known from the type locality, growing under *P. patula.*

**Diagnosis:**—The new species differs from other species within the Maculatum clade by its pale brown ascomata, finely granular peridium, composed of subglobose hyphae, forming pyramid-like structures, clavate terminal cells of 11−30 × 6−10 µm, pale brown to gray gleba, and 22−52 × 15−40 µm alveolate ascospores.

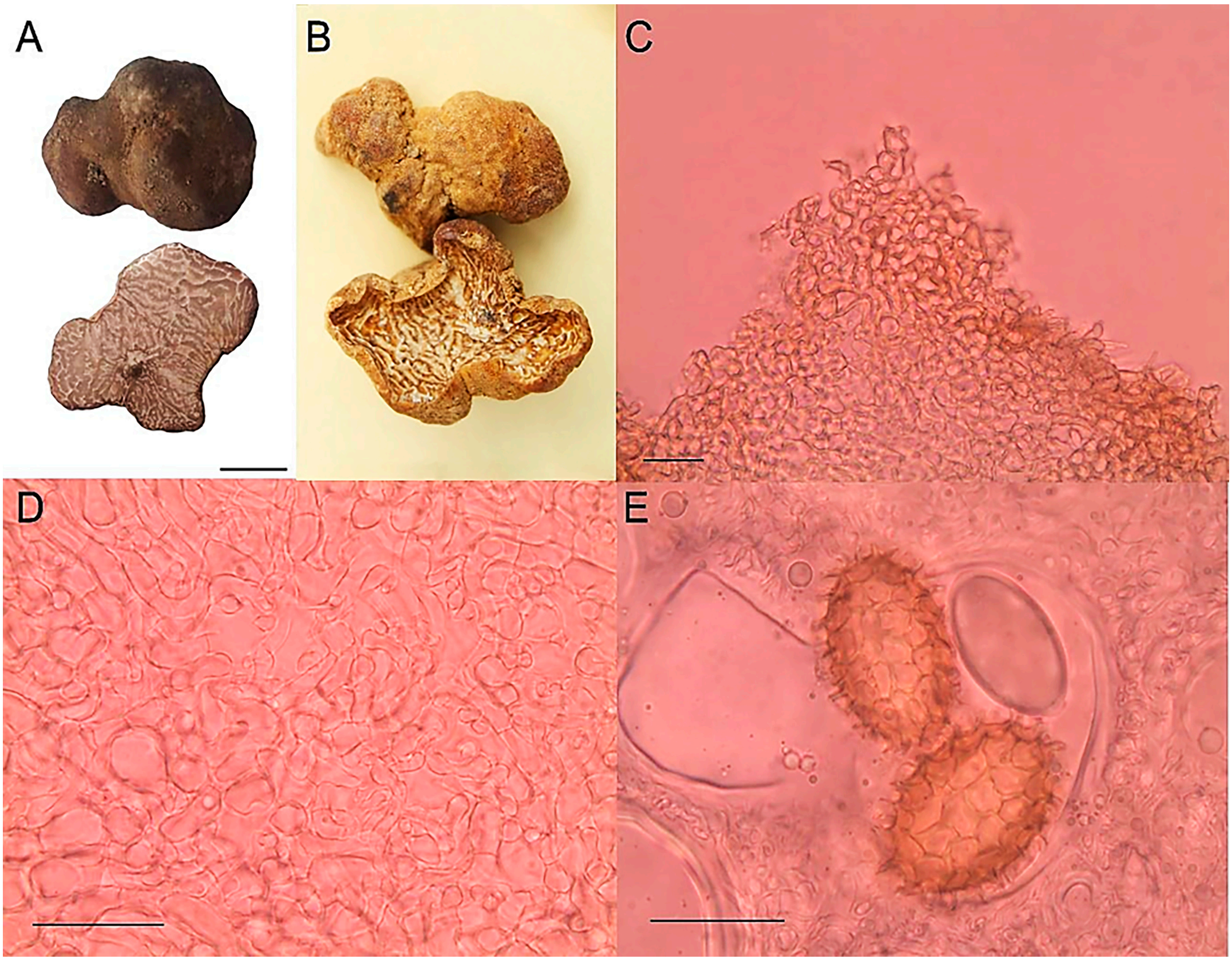

**FIGURE 2**. *Tuber itzcuinzapotl* (Holotype). **A**) Fresh ascomata. **B**) Dry ascomata. **C**) Peridium hyphal arrangement. **D**) Hyphae from sterile veins. **E**) 3-spored ascus containing 2 mature ascospores showing alveolate ornamentation. Bar: **A**–**B**= 10 mm; **C**–**E**=20 µm.

## Discussion

Analysis of the ITS region places the new species within the maculatum clade, which is characterized by having species with granular or cottony peridium, clavate elements on the peridium, and honeycomb ascospores (Montecchi & Sarasini 2000; Bonito *et al*. 2013). The species of this clade are located mainly in the northern hemisphere, being present in the United States and Mexico (García-Jiménez *et al*. 2021). A synopsis of the diagnostic characteristics of the Mexican *Tuber* species related to the new species described in this work, including their host and distribution is presented in the Table 2. Even though the species in the maculatum clade are not highly appreciated due to their inconspicuous taste and smell, some species such as *T. miquinuanense* could be commercialized (Guevara-Guerrero *et al*. 2022). Most of the species within this clade are associated with trees of the genera *Abies*, *Carya*, *Pinus* and *Quercus* (Sánchez-Ledezma *et al*. 2023). With the addition of the new species described in this study, a total of 26 species belonging to the genus *Tuber* have been reported from Mexico, mostly distributed in northeastern Mexico (Figure 3).

**TABLE 2.** Diagnostic characteristics of the new species described in this work compared to related species.

| Character | *T. aztecorum* | *T. castilloi* | *T. itzcuinzapotl* | *T. miquihuanense* |
|---|---|---|---|---|
| Ascomata | | | | |
| Size | 5–23 × 4–16 × 3–11 | 11–25 × 10–17 | 35 × 28 | 30–20 |
| Shape | Subglobose, irregular, lobate or globose | Subglobose to ovoid, flattened or irregular | Subglobose irregular to lobate | Subglobose to slightly gibbose |
| Texture | Finely verrucose or granulose | Minutely verrucose to papillose | Finely verrucose or granulose | Flat-polygonal to pyramidal warts |
| Taste | Not recorded | Not recorded | Fruity | Not recorded |
| Smell | Raw potato-like | Not recorded | Fruity | Pleasant |
| Peridium | | | | |
| Width (µm) | 110–350 | 80–150 | 200−400 | 110–360 |
| Color | Hyaline to reddish brown | Hyaline to reddish brown | Hyaline to reddish | Yellowish to reddish brown |
| Hyphal arrangement | Pseudoparenchima | Pseudoparenchima | Pseudoparenchima | Pseudoparenchima |
| Gleba | | | | |
| Appearance | Marbled | Marbled | Marbled | Marbled |
| Color | White to greyish | Light brown | Pale brown to greyish brown, dark brown when mature | Cream to light brown |
| Hyphal arrangement | Interwoven | Interwoven to periclinal, | Interwoven | Interwoven to periclinal |
| Asci | | | | |
| Size | 62–95 × 57–77 | 90 × 50–65 | 34−70 × 20−50 | 65–90 × 51–70 |
| Shape | Globose, subglobose to broadly ellipsoid | Globose to subglobose or ovoid, some with a short pedicel | Globose to subglobose, hyaline, some with a globose pedicel | Globose to subglobose or broadly ellipsoid, with a short pedicel when young |
| Number of spores | 1 to 4 | 1 to 4 | 1 to 4 | 1 to 5 |
| Ascospores | | | | |
| Shape | Subglobose, globose to broadly ellipsoid | Subglobose to broadly ellipsoid | Ellipsoid | Globose to subglobose or broadly ellipsoid, with a short pedicel when young |
| Size in spores per asci (µm) * | 1) 42–58 × 27–48; 2) 25–52 × 23–40; 3) 27–40 × 20–30; 4) 23–38 × 18–28 | 1) 27–63 × 20–40; 2) 30–50 × 23–34; 3) 27–40 × 20–32; 4) 27–44 × 20–30 | 1) 40−52 × 23−30; 2) 27−34 × 15−22; 3) 22−39 × 15−19; 4) 25−42 × 13−30 | 1) 40–50 × 30–39; 2) 29–40 × 24–35; 3) 24–35 × 22–33; 4)23–28 × 20–26; 5) 20–28 × 20–22 |
| Changes of color | None | None | None | None |

......*continued on the next page*

**TABLE 2.** (Continued)

| Character | *T. aztecorum* | *T. castilloi* | *T. itzcuinzapotl* | *T. miquihuanense* |
|---|---|---|---|---|
| Habit and habitat | Hypogeous, gregarious in volcanic rock soil in an *Abies religiosa* forest | Hypogeous, solitary to scattered. | Hypogeous, solitary. | Hypogeous, solitary to scattered. |
| Type of vegetation | *Abies religiosa* forest | Mixed forest with *Quercus* spp. and *Pinus* spp. | *Pinus* forest | *Pinus*-*Quercus* and mesophyll forests |
| Geographical distribution | State of Mexico, Mexico. | Nuevo León and Tamaulipas, Mexico. | Veracruz, Mexico. | Tamaulipas, Mexico. |
| Reference | Guevara-Guerrero *et al.* 2018 | Guevara-Guerrero *et al.* 2013 | This work | Guevara-Guerrero *et al.* 2013 |

* the number before parenthesis indicates the number of spores per asci

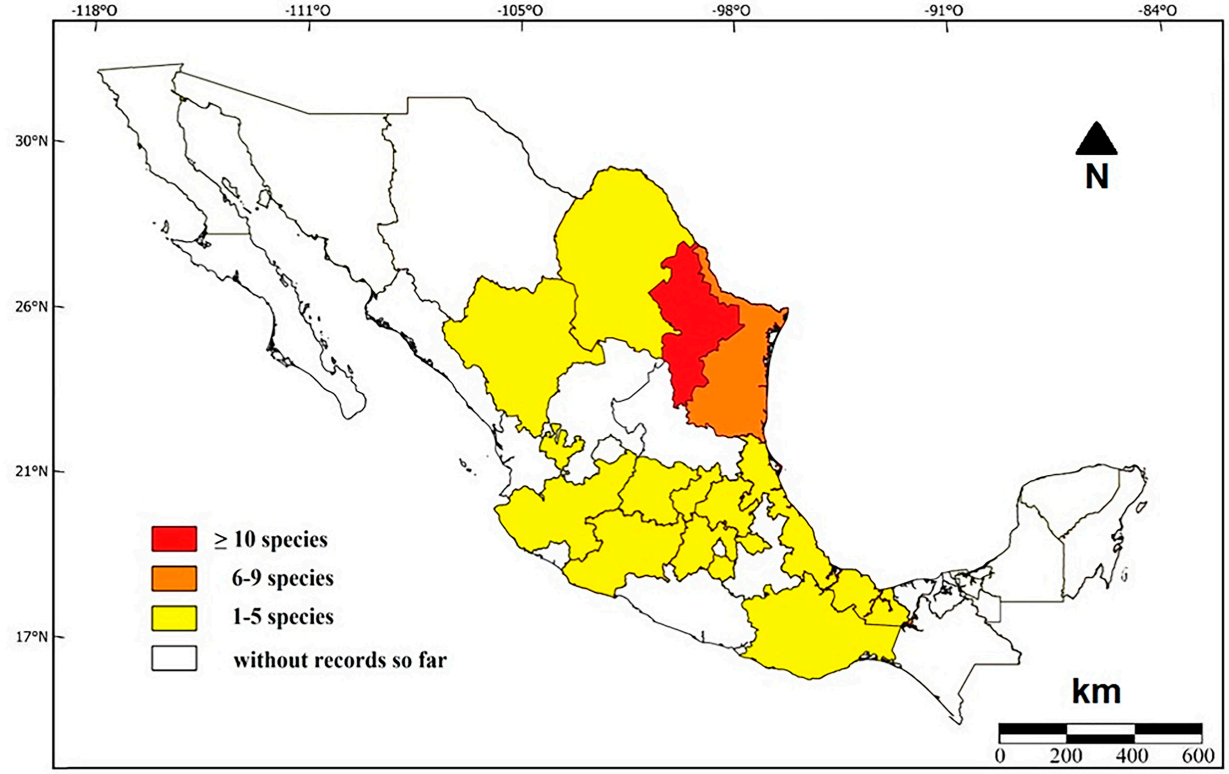

**FIGURE 3**. Heatmap showing the distribution of the recorded *Tuber* species in Mexico.

*Tuber itzcuinzapotl* is mainly characterized by a light brown, verrucous-granular peridium, gray or pale brown gleba, peridium formed by pseudoparenchymatous hyphae with 11−30 × 6−10 µm clavate elements and 22−52 × 15−40 µm ascospores. Like the rest of the species of the maculatum clade, *T. itzcuinzapotl* has a pseudoparenchymatous peridium with clavate terminal elements and alveolate ascospores. It resembles to *T. miquihuanense*, since they are similar in the thickness and color of the peridium, including the size of the ascospores. However, these two species phylogenetically belong to a different clade and morphologically they can be distinguished by its peridial hyphal microscopic structure, being composed of erect hyphal chains in *T. miquihuanense* (Guevara-Guerrero *et al.* 2012) and pyramidal-shaped hyphae in *T. itzcuinzapotl.* Another similar species is *Tuber mexiusanum* which phylogenetically is a sister species of *T. miquihuanense,* and differs from *T. itzcuinzapotl* by having ascomata with a strong greenish reaction when cut, strong acetone-like smell and larger terminal cells in the peridium (Guevara-Guerrero *et al.* 2013a).

Mexico constitutes one of the main biocultural centers of edible wild fungi in the world. In the country around 500 species are consumed by the 71 ethnic groups and by the rural communities which inhabit areas nearby the forested areas. The country is ranked therefore as the second international genetic center of edible wild fungi, just after China, where more than 1000 species of fungi are consumed (Pérez-Moreno *et al.* 2021a; 2021b). Paradoxically, most of the species which are consumed in Mexico are epigeous and only a very reduced number of hypogeous fungi are consider a food resource. Currently, only two species of the genera *Melanogaster* Corda and *Gautieria* Vittad. have been reported as edible by different ethnic groups in Mexico (Cázares *et al.* 2008; Montoya *et al.* 2012). Due to the fact that the country holds the largest diversity of oaks in the world, with more than 160 species, and 72 taxa of pines, which are trees that establish ectomycorrhizal symbiosis with a high number of hypogeous fungi, is reasonably assume that in general these group of fungi have been historically overlooked.

*Tuber itzcuinzapotl* is consumed in the central area of the Mexican state of Veracruz, located in eastern Mexico, by members of the Nahua culture from the Sierra de Zongolica, where it is known as itzcuinzapotl (dog's *zapote*). Trying to trace the origin of these name of Nahuatl origin we learnt that according to local people, the name is related to the fact that in the past local peasants were accompanied by dogs while conducting agricultural practices. And these dogs use to dig up searching for this truffle; later on, at a time that is lost in time, people realize that they this truffle was edible for humans as well, and they start enjoying their flavor. They named this truffle *itzcuinzapotl*, as it is called at the present time. The word "Izcuintli", comes from the Nahuatl, the language used by the Aztec people before the conquest of Mexico by the Spaniards. It was used to designate a native hairless dog breed, often sacrificed in sacred rituals during ancient times throughout Mexico, and then buried with their owners, believing they acted as guides to the soul on its journey to the underworld. Meanwhile, the word "Zapotl" comes from the resemblance of the smell, color, taste and shape of the new species of truffle with a local fruit known as "zapote". Currently, peasants no longer use dogs when they carry out their agricultural practices, and therefore the collection of *Tuber itzcuinzapotl* is carried out randomly when they prepare the soil near forested areas for planting milpa (an ancient Mesoamerican agricultural system which is used to produce maize, beans, and squash without employing artificial pesticides or chemical fertilizers). Nahua people of this area enjoy eating this truffle, which is consumed raw when found in their mature stage. Its phenology is included in the category known technically as "short at the middle of the rainy season" during the months of June to August. Interestingly, the inhabitants do not include *T. itzcuinzapotl* in the category of fungi, but they consider it as a fruit. The community of Mexcala, where the species was found, has 1,883 inhabitants (INEGI, 2020), among which we only detected three women aged 50 to 70 years with a deep traditional knowledge of this fungus, who additionally consume regularly *T. itzcuinzapotl*. Therefore, the accurate traditional knowledge related to the ecology, distribution, phenology, and consumption is entirely associated with adult women, who are responsible for preparing the soil for planting, indicating the huge cultural erosion and the high risk of disappearance of the traditional knowledge of this species. Due to the great bio-cultural relevance of this truffle species, it would be very relevant to develop further studies related to the determination of its nutrition contents and the identification of its potential bioactive compounds with medicinal properties.

Therefore, this contribution reports the first edible truffle with traditional biocultural importance among ethnic groups in Mexico. The contribution intends to incentivize the interest, development, and rescue of traditional knowledge of the consumption of hypogeous fungi in Mexico. At the same time, it is intended to mitigate the mycocultural erosion that has been happening in a short period of time, particularly in the last three decades, at a quick pace, as never recorded before, not only in Mexico but on a global scale.

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