

Tuber regimontanum, new species of truffle from Mexico

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Tuber regimontanum, una nueva especie de trufa de México

Resumen. Se describe *Tuber regimontanum* como especie nueva del norte de México (Nuevo León). Esta especie es afín a *T. melanosporum* y *T. indicum*, pero estas dos especies presentan esporas mas pequeñas que la especie que se describe. Molecularmente y basandose en un análisis del ITS ribosomal estas tres especies difieren significativamente. Con la nueva especie son ya seis las especies de *Tuber* conocidas de México.

Palabras clave: Hongos hipogeos, *Quercus*, *Tuber*.

Abstract. *Tuber regimontanum* is described as a new species from the N of Mexico (Nuevo Leon). It is close to *T. melanosporum* and *T. indicum* but these species have smaller spores than the described species. Molecular analysis of the the ITS ribosomal has shown these threes species are significatly different. With the new species are until now six *Tuber* species known from Mexico.

Key words: Hypogeous fungi, *Quercus*, *Tuber*.

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Introduction

In Mexico only five species of *Tuber* are known (Table 1) and two undescribed. In August of 2007 several black truffles in the genus *Tuber* were collected in a *Quercus* forest in the region of Sierra of Picachos in Nuevo León. Due to the uncertainty of its taxonomic affiliation to either *T. melanosporum* or *T. indicum*, a ribosomal DNA study was performed. We conclude that this is a new species and we have described its morphological characteristics and phylogenetic placement.

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Materials and methods

The methods of Gilkey (1939, 1954), Castellano *et al.* (1989) and Pegler *et al.* (1993) were used for their morphological description. Hand cut sections were mounted in KOH 5% and Melzer reagent for microscopic examination. Compound microscope, stereoscope and Scanning Electron Microscope (SEM) were used for the microscopic analysis. For molecular analyses DNA was extracted with CTAB 2x buffer using the chloroform extraction technique. The ribosomal ITS and LSU regions were PCR amplified using a standard set of primers (ITS5 & LR5) and thermocycle conditions (Bertini *et al.* 1999; Gardes *et al.* 1991; Vilgalys & Hester, 1990). Four µl of each PCR product was loaded into a 1.0% agarose gel buffered with TAE buffer and stained with 2 µl SYBR safe (Invitrogen, Carlsbad, CA) per 80 ml TAE buffer. Gel

Table 1. *Tuber* species known from Mexico

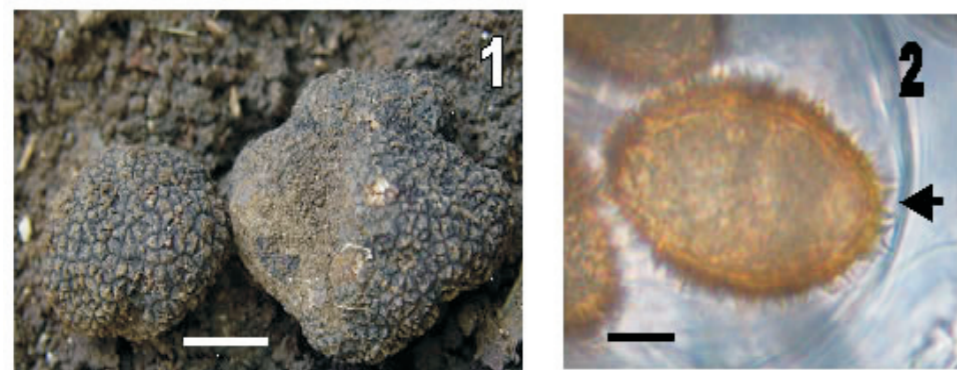
Specie	Locality	Reference
<i>T. gardneri</i> Gilkey as <i>T. murinum</i> Hesse	Coahuila, Mexico, Nuevo Leon and Morelos	Trappe & Guzmán 1971; Cázares <i>et al.</i> 1992
<i>T. guzmanii</i> Trappe & Cázares	Morelos	Trappe & Cázares 2006
<i>T. lyonii</i> Butters as <i>T. rufum</i> var. <i>nitidum</i> (Vitt.) Fisher or <i>T. candidum</i> Hark	Nuevo Leon, Mexico, Tamaulipas	Cázares <i>et al.</i> 1992 ; Garza <i>et al.</i> 1985; Trappe & Cázares 2006; García & Guevara 2005
<i>T. maculatum</i> Vitt.	Nuevo Leon	Cázares <i>et al.</i> 1992
<i>T. separans</i> Gilkey	Nuevo Leon	Cázares <i>et al.</i> 1992
<i>Tuber</i> sp.	Puebla	Trappe & Cázares 2006
<i>Tuber</i> sp.	Veracruz	Trappe <i>et al.</i> 1996

electrophoresis products were viewed on a GelDoc XR imager (Bio-Rad Laboratories, Inc., Hercules, CA). PCR products were cleaned using Qiagen Quick-Clean columns and used for PCR sequencing reaction. Sanger sequencing was performed using Big Dye chemistry version 3.1 (Applied Biosystems, Foster City, CA) with either the primer ITS5 (forward) or LR5 (reverse). DNA sequences were determined on an ABI3700 (Applied Biosystems, Foster City, CA). Unambiguous regions were excluded from the final ITS alignment and the dataset was analyzed using unweighted parsimony methods in PAUP 4.0b10 (Swofford, 2001), using 1000 random addition sequences and 5000 bootstrap replicates. The North American species *T. lyonii* Butters, was chosen as an outgroup based on preliminary data on phylogenetic relationships across the genus *Tuber*. The ITS and LSU sequences of *T. regimontanum* and *T. lyonii* produced in this study were deposited in the GenBank under the access number EU375838 and EU394704, respectively.

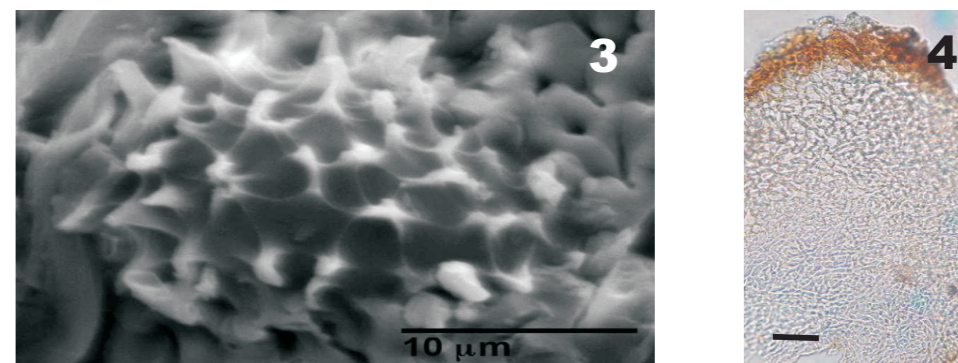
Results

Tuber regimontanum Guevara, Bonito & Rodríguez sp. nov
Figs. 1-5

Ascomata 10-40 x 8-18 mm, *globosa, subglobosa vel lobata, atrobrunnea vel nigra, verrucis pyramidalibus usque ad 2-3 mm latis et 1-1.5 mm altis. Gleba solida, maturitate atrobrunnea vel nigra venis albis marmoratis. Peridium* 100-300 μ m *crassum, epicute pseudoparenchymatisque subcute intertextis. Sporae fusiformes vel ellipsoideae, 33-55 (-62) x 23-31 μ m, maturitate porphyrae, spinis 2-5 x 1-2 μ m. Asci globosi, subglobosi vel ovoidei, hyalini, acaules, sporis 1-4. Species interjecta *Tuber melanosporum* et *T. indicum*. (*Holotypus* Guevara 909, *ITCV* hic designatus).*



Figuras 1-2. *Tuber regimontanum*. 1: Ascomata (bar = 1 cm), 2: Asci with 3 ascospores, the arrow indicates the spines (bar = 10 μ m).



Figuras 3-4. *Tuber regimontanum*. 3: Ascospore, microphotograph SEM of the surface. 4: Peridium, cross section showing the pseudoparenchima toward the exterior and the interwoven cells in the interior (bar= 50 μ m).

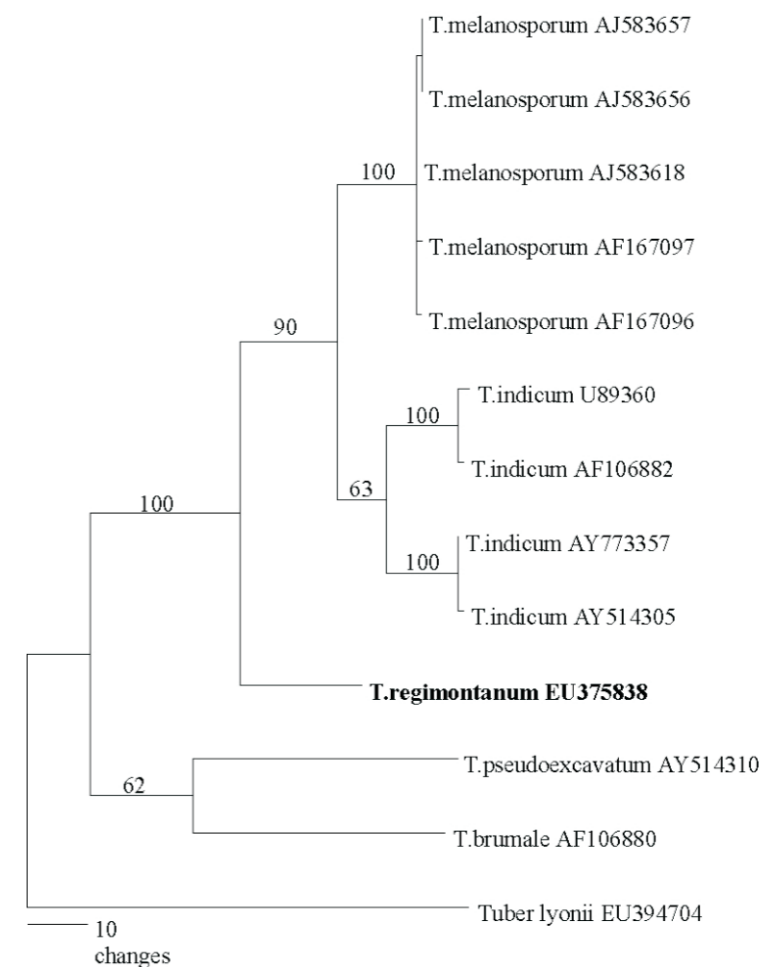


Figura 5. Phylogenetic placement of *Tuber regimontanum*: The single most parsimonious tree of the black truffles (*Tuber melanosporum* clade) based upon ITS rDNA. Bootstrap values are presented on the branch nodes. Taxa are labelled by their collection name and GenBank accession number.

Ascomata 10-40 x 8-17 x 8-18 mm, *globose, subglobose to lobulated, dark brown to black, dry surface, rough with pyramidal verrucae 2-3 mm of wide x 1-1.5 mm of high,*

without basal mycelium or rhizomorphs. Gleba solid, white to light brown or dark, black when dried, with white veins toward the peridium, marbled. Odor fragrant and pleasant,

flavor fungi-like. KOH 5% and FeSO₄ 10% negative or lightly black after 10 min. Peridium 100-300 µm thick. Outer layer a pseudoparenchyma of 100-250 µm wide, hyphae 5-30 µm diam. angular or isodiametric, reddish brown to dark coffee, thick walled, without intracellular contents. Inner layer of 30-50 µm thick, difficult to distinguished from the outer layer, interwoven hyaline hyphae. Veins formed by hyaline interwoven hyphae. Ascospores 33-55 (-62) x 23-31 µm without ornamentation, reddish brown; 1 spore in an asci are 40-55 (-62) x 30-31 µm, with two 37-42 x 25-26 µm, with three 33-37 x 23-26 µm, with four 28-35 x 18-22 µm, broadly fusiform to ellipsoid. Spines 2-5 x 1-2 µm, thin, robust toward the base, hyaline in youth, straw yellow to reddish brown when mature, thick walled. Asci globose to subglobose or ovoid, without pedicel, thick walled, 2-5 µm thick, hyaline.

Habitat. Hypogeous, gregarious, under a *Quercus polymorpha* forest, on the side of intermittent stream, soil pH 8.

Collections examined. Nuevo León, Sierra of Picachos, Mpio. de Higuera, August 23, 2007, *Guevara 909* (Holotype ITCV, isotypes OSU and DUKE).

Discussion. *Tuber regimontanum* is similar macro- and microscopically to the European species *T. melanosporum* Vitt. and the Chinese species *T. indicum* Cooke & Masee. *T. melanosporum* has spores 28-32 x 16-21 µm, blackish brown, while *T. indicum*, 26 x 17 µm, brown-blackish (Montecchi & Sarasini, 2000). The molecular results using the ITS nuclear ribosomal DNA, demonstrated that *T. regimontanum* is significantly different from these two species at the molecular level (Fig. 5).

Etymology: Latin, *regi-* (region) and *montanum* (mountain), from the "mountainous regions" with respect to the Cd. of Monterrey where its citizens are denominated "regimontanos".

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