Advances in the phylogeny of the genus Tulostoma in Mexico, new records for the country, Jalisco and Zacatecas

Avances en la filogenia del género Tulostoma en México, nuevos registros para el país, Jalisco y Zacatecas

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Abstract
Fungi of the genus *Tulostoma* are part of the diverse order Agaricales. These fungi comprise a monophyletic group characterized by globose-stipitate basidiomes, a powdery gleba, and a stipe with a volva or volvoid structure. *Tulostoma* is considered cosmopolitan; however, little is known about its species diversity and phylogenetic relationships in the Neotropics region. We used Tulostoma specimens from Mexico to assess the global diversity of the *Tulostoma* genus and the phylogenetic relationships of Neotropical *Tulostoma* species. DNA sequences of two regions, the internal transcribed spacer region (ITS) and the 28S nuclear subunit (LSU), were used to construct a phylogenetic hypothesis and discuss the phylogenetic relationships with Paleotropical and Paleartic *Tulostoma* species. A total of 224 sequences of *Tulostoma* were analyzed, including 16 new sequences from Mexican specimens and one from Spain. The phylogenetic results, which considered both morphological traits and the ITS and LSU sequences, did not support the previous identification of most of the 16 Mexican specimens of *Tulostoma*, which was based on morphological traits. The phylogenetic analysis validated a new report for Mexico *Tulostoma rufum*, and three new records of *Tulostoma* for western Mexico (*Tulostoma fimbriatum*, T. *striatum* and *T. xerophilum*) from paleotropics and palearctics present in the Neotropical region, wish shows the high diversity of the genus in this region that has been little studied. Our study contributes to the knowledge of the *Tulostoma* genus, which includes species considered pseudocryptic due to their high morphological variability. Moreover, our study emphasizes the need for molecular techniques to support taxa determination.

Keywords: Agaricaceae, Agaricales, ITS, Molecular phylogeny, Neotropical, nucLSU.

Resumen
Los hongos del género *Tulostoma* forman parte del orden Agaricales. Estos hongos comprenden un grupo monofilético caracterizado por basidiomas globosos-estipitados, una gleba pulverulenta y un estípite con una volva o estructura volvoide. *Tulostoma* se considera cosmopolita; sin embargo, se sabe poco sobre su diversidad de especies y relaciones filogenéticas en la región neotropical. Utilizamos especímenes de *Tulostoma* de México para evaluar la diversidad global del género *Tulostoma* y las relaciones filogenéticas de las especies neotropicales. Se utilizaron secuencias de ADN de dos regiones, la región espaciadora transcrita interna (ITS) y la subunidad nuclear 28S (LSU), para construir una hipótesis filogenética y discutir las relaciones filogenéticas con especies de *Tulostoma* paleotropicales y paleárticas. Se analizaron un total de 224 secuencias de *Tulostoma*, incluidas 16 secuencias nuevas de especímenes mexicanos y una de España. Los resultados filogenéticos, que consideraron tanto caracteres morfológicos como las secuencias ITS y LSU, no respaldaron la identificación previa de la mayoría de los 16 especímenes mexicanos de *Tulostoma*, que se basó en caracteres morfológicos. El análisis filogenético validó un nuevo reporte de *Tulostoma* para México, *T. rufum*, y tres nuevos registros de *Tulostoma* para el occidente de México (*Tulostoma fimbriatum*, T. *striatum* y T. *xerophilum*) paleotropicales y paleárticas presentes en la región neotropical, lo que evidencia la alta diversidad del género en esta región que ha sido poco estudiada. Nuestro estudio contribuye al conocimiento del género *Tulostoma,* que incluye especies consideradas pseudocrípticas debido a su alta variabilidad morfológica. Además, nuestro estudio enfatiza la necesidad de técnicas moleculares para apoyar la determinación de taxones.

Palabras clave: Agaricaceae, Agaricales, Filogenia molecular, ITS, Neotropical, nucLSU.

Introduction

Tulostoma Pers. is a group of fungi that belongs to Agaricaceae in the order Agaricales, commonly referred to as stalked-puffballs or tulostomataceous. This genus is characterized by a spore-sac surrounded by peridium, a hollow stipe, a pulverulent gleba, and the presence of a volva or a volvoid structure (Wright, 1987). Spore dispersion occurs via powdery gleba puffing from the mouth, an apical ostiole, or an irregular opening due to external pressure on the peridium. The powdery gleba consists of a capillitium and ornamented or smooth spores (Moreno *et al*., 1995). The genus *Tulostoma* comprises 139 species based on macro and micro characters, as described in the world monograph by Wright (1987). Currently, Index Fungorum (2024) recognizes 173 species, of which only 59 are fully or partially sequenced and deposited at the GenBank nucleotide database. *Tulostoma* has a worldwide distribution and is generally found in arid regions with sandy, clayey soils, as well as forests, pastures, roadsides, and temperate and tropical habitats (Wright, 1987).
From a morphological standpoint, the *Tulostoma* genus has been extensively studied using macro- and micro-morphological traits; optical and scanning electron microscopy have been used for species identification (Moreno *et al*., 1992, 1997; Altés & Moreno 1995, 1999. This approach is consistent with Wright's (1987) morphological species concept and with the correlation of environmental factors or ecological interactions that have allowed taxa segregation. However, Tulostoma morphology is highly variable, complicating the identification of different species. Recent studies include DNA sequences in their analyses to recognize new species (Hussain *et al*., 2016; Rehman-Niazi *et al*., 2022) or estimate the genetic diversity of certain taxa (Rusevsk *et al.*, 2019; Kumar-Dutta *et al*., 2020).
Jeppson *et al*. (2017) have recognized the *Tulostoma* genus as a monophyletic group. They found that many morphological characters previously used to segregate species were actually plesiomorphic or homoplastic. They also observed that the ornamentation of spores and the hyphal structure of the peridium are informative characters for species delimitation. Their study provided sequences of 34 holotypes and characterized 30 known species, indicating that the diversity of *Tulostoma* species is much higher than previously thought, especially with European specimens. However, only a few studies in the Neotropical region have included molecular data, including the reports of the new species *Tulostoma domingueziae* from Argentina and *T. rufescens* from Mexico, by Hernández-Caffot *et al*., (2011) and Hernández-Navarro *et al*.(2018), respectively.
In this study, we used DNA sequences to understand the global diversity of the *Tulostoma* genus and the phylogenetic relationships of the Neotropical *Tulostoma* species. In particular, we used Mexican *Tulostoma* specimens and DNA sequences of two regions, ITS and LSU, to construct a phylogenetic hypothesis and discuss their phylogenetic relationships with the Paleotropical and Paleartic *Tulostoma* species.

Material and methods
Morphological data

We reviewed *Tulostoma* specimens from the Mycological Collection at the IBUG Herbarium of the Universidad de Guadalajara. The Mexican collections came eight of them from the Nearctic region and nine from the Mexican Transition Zone, according to the biogeographic regionalization in Mexico as indicated by Morrone (2019). Seventeen specimens were selected, one from Spain and 16 from Mexico. Mature basidiomata of *Tulostoma* were studied under a stereo-microscope, regarding their macromorphological traits (spore-sac, mouth, peristome, exo and endoperidium, socket, stipe), in accordance with Wright (1987). Micromorphological analyses were performed using light microscopy (LM); Zeiss K-7 microscope (Jena, Germany) to observe features as like as the gleba (spores and capillitium) mostly, and for this preparation were made in KOH (5%). Studies under scanning electron microscopes (SEM); EVO-50 Zeiss were conducted according to the procedure of Moreno *et al*., (1995). Specialized literature, including Calonge (1998), Wright (1987), Moreno *et al*., (1995), Esqueda *et al*., (2004, 2012), and Hernández-Navarro *et al*., (2020), was consulted to identify the species. The dried collections examined were photographed ex situ in the laboratory, and the photographs of the microscopic characters, an optical microscope (Zeiss Axioscop 40, Jena, Germany), camera (AxioCam MRc, Zeiss, Jena, Germany) and Axio Vision 4 software (Carls Zeiss Microscopy) were used.

DNA extraction, PCR, and sequencing

DNA was extracted from the herbarium species using the CTAB protocol with some modifications (Doyle & Doyle, 1987). Specimens no larger than 1 cm^2 were carefully taken from the peridium avoiding damage to the apical ostiole. DNA quality and quantity was assessed by electrophoresis and spectrometry with a NanoDrop 2000 (Thermo Scientific).
To amplify the interspacer region of nuclear ribosomal RNA (nrITS), we used the specific primers ITS1 and ITS4 (White et al., 1990); for the Large Subunit (LSU), we used

the primers LROR and LR6 (Hopple & Vilgalys, 1999). Each PCR reaction was adjusted to a volume of 25 µL using the following reagents: 16.375 µL of HPLC water, 5 µL of colorless Go-Taq® buffer, 0.5 µL of mix dNTPs (5 mM), 0.5 µL of each primer (10 µM), 0.125 µL of Go-Taq® polymerase, and 2 µL of DNA (~20 ng/µL).

The PCR conditions for ITS were as follows: an initial denaturation cycle at 95ºC for 3 min; 35 cycles with three steps, starting at 95ºC for 1 min, an alignment temperature of 54.8ºC for 45 s, and 72ºC for 2 min; a final cycle at 72ºC for 10 min; and an incubation phase at 4ºC. For LSU, the conditions were as follows: an initial denaturation cycle at 95ºC for 4 min; 35 cycles with three steps, starting at 94ºC for 1 min, an alignment temperature of 58ºC for 1 min, and 72ºC for 1 min; a final cycle at 72ºC for 10 min; and an incubation phase at 4ºC. We confirmed the amplification by electrophoresis in 1% agarose gels.

The PCR products were purified with the ExoSAP IT enzyme. The sequencing reaction was performed with the BigDyeTM Terminator v3.1kit and purified. The sequences were obtained with SeqStudio Genetic Analyzer (Applied Biosystems). The forward and reverse sequences were assembled using Sequencher 4.9 (Gene Codes, Ann Arbor, MI, USA) and manually aligned with PhyDe (Miller *et al*., 2010). The sequences reported in this paper are available at GenBank (accession numbers are listed in Table 1). Additionally, sequences of ITS and LSU for the ingroup and outgroup taxa were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank/; see Annex 1). Genomic DNA extraction, product amplification, purification, protection of specimens, and sequencing were performed at the Laboratorio Nacional de Identificación y Caracterización Vegetal (LaniVeg), Universidad de Guadalajara.

Table 1. Data of the Tulostoma Mexican species studied.

Taxon	GenBank Accession	Voucher	Location	Collection date
Tulostoma fimbriatum	OR594177	SA7	Jalisco, Mexico	20/10/2017
Tulostoma rufum	OR594166	DFG420	Jalisco, Mexico	27/07/2019
Tulostoma striatum	OR594178	OR2302	Jalisco, Mexico	30/08/2000
Tulostoma xerophilum	OR594173	AF	Zacatecas, Mexico	08/2017
Tulostoma sp. 1	OR594162	OR2436	Madrid, España	25/01/2002
Tulostoma sp. 2	OR594163	JAPR1945	Coahuila, Mexico	03/08/2006
Tulostoma sp. 3	OR594164	OR2321	Jalisco, Mexico	30/08/2000
Tulostoma sp. 4	OR594165	PCR1414	Querétaro, Mexico	19/11/2000
Tulostoma sp. 5	OR594169	ICCM366	Jalisco, Mexico	08/06/2004
Tulostoma sp. 6	OR594167	PB7	Jalisco, Mexico	17/07/1998
Tulostoma sp. 7	OR594168	OVB17	Jalisco, Mexico	17/07/1994
Tulostoma sp. 8	OR594170	SS30	Jalisco, Mexico	04/07/1999
Tulostoma sp. 9	OR594171	OR1816	Jalisco, Mexico	07/09/1997
Tulostoma sp. 10	OR594172	OR2308	Jalisco, Mexico	30/08/2000
Tulostoma sp. 11	OR594174	GEDR12	Jalisco, Mexico	10/2017
Tulostoma sp. 12	OR594175	NA	Nuevo León, Mexico	04/09/2001
Tulostoma sp. 13	OR594176	CO77	Zacatecas, Mexico	17/03/2017

Phylogenetic analysis

Our sampling included 224 DNA sequences; of these, 16 were newly generated from Mexican *Tulostoma* species, one was from Spain, and 205 were used previously in the phylogenetic analysis of *Tulostoma* (Jeppson *et al*., 2017). *Lycoperdon subcretaceum* was used as the functional root. First, we used ModelTest-NG (Darriba *et al*., 2020) to identify the molecular evolution model that best fit our two different matrices. Next, we performed Maximum Likelihood (ML) analyses using RAxML v. 8 (Stamatakis, 2014) and implemented through raxmlGUI v 2.0.10 (Edler *et al*., 2021). Since our nrDNA matrices were incomplete (see Annex 1, we used ML to assess how well the method reconstructed the topology with incomplete data (Wiens & Morrill, 2011; Jiang *et al.*, 2014). Nodal support was estimated with a parametric bootstrap with 1000 replicates. Because most of the *Tulostoma* sequences came from the Jeppson *et al*., (2017) study, we followed their phylogenetic hypothesis recognizing 11 clades.

Results

Based on the morphological characters observed macroscopically and micromorphological analyses (LM, SEM) of the 17 specimens studied, one of them, *Tulostoma rufum* (OR594166) is new report for Mexico, two are described here as new records from Jalisco *Tulostoma fimbriatum* (OR594177); *Tulostoma striatum* G. Cunn. (OR594178) and one of Zacatecas *Tulostoma xerophilum* Long. (OR594173). The rest of the specimens (8) could not be determinated, so they were left only up to genus *Tulostoma* sp.

The total number of aligned base pairs (bp) for the ITS and LSU was 2269 bp. The molecular evolution model generated by the ModelTest-NG analyses for the concatenated matrix was GTR+I+G. Our phylogenetic tree (Fig. 1) contained 11 clades, with the bootstrap supports (BS) written below the nodes and shown in Figs. 1-4. Our specimens were recovered in clades 3, 5, 7, 9, 10, and 11, and two of them (*Tulostoma* sp. 10, *Tulostoma* sp. 11) were not assigned to any (Fig. 1). In our tree, *Tulostoma xerophilum* OR594173 was recovered as a sister to holotype material of *T. xerophillum* KX576549 with 100% BS, and is validated as a new record for Jalisco, while the clade formed by *Tulostoma* sp. 3 and *Tulostoma* sp. 6 was sister to this clade with 87% BS. The relationships between clades had 100% BS, and *Tulostoma* sp. 2, *Tulostoma* sp. 5 and *Tulostoma* sp. 12 were all recovered in clade 10 (Fig. 2). *Tulostoma* sp. 2 and *Tulostoma* sp. 5 were sister taxa with 96% BS, and *Tulostoma* sp. 12 was sister to them with 74% BS.

However, the position of this clade was not resolved (Fig. 2). *T. rufescens* and *Tulostoma* sp. 10 KU519065 were recovered as sister taxa, with *Tulostoma* sp. 4 sister to them with 97% BS, all in clade 10 (Fig. 2). Finally, *Tulostoma striatum* OR594178 was recovered in clade 9, being the sister species to the clade formed by two T. striatum specimens (Fig. 2) with 93% BS considered as a new report for Jalisco. In clade 7 (Fig. 3), *Tulostoma* sp. 1 and various sequences of *Tulostoma* sp. were the sister species of *T.* cf. *submembranaceum*, with 66% BS. In clade 5, *Tulostoma* sp. 7 was sister to *Tulostoma* sp. 8 with 65% BS. *Tulostoma* sp. 9 was sister to *T. lusitanicum*, but without BS (Fig. 3). *Tulostoma fimbriatum* OR594177 was nested in clade 3, close to the *T. fimbriatum* clade (Fig. 4). *Tulostoma* sp. 13 was the sister species of *Tulostoma* sp. 3 (*T.* cf. *fimbriatum* KU518978) with 100% BS, also in clade 3 (Fig. 4). In clade 11 (Fig. 4), *Tulostoma rufum* OR594166 was sister to *T. rufum* with 100% BS. *Tulostoma* sp. 11 was the sister to clades 3 and 11 with 51% BS, and *Tulostoma* sp. 10 was sister to taxon *Tulostoma* sp. 11 and clades 3 and 11, but without BS (Fig. 4).

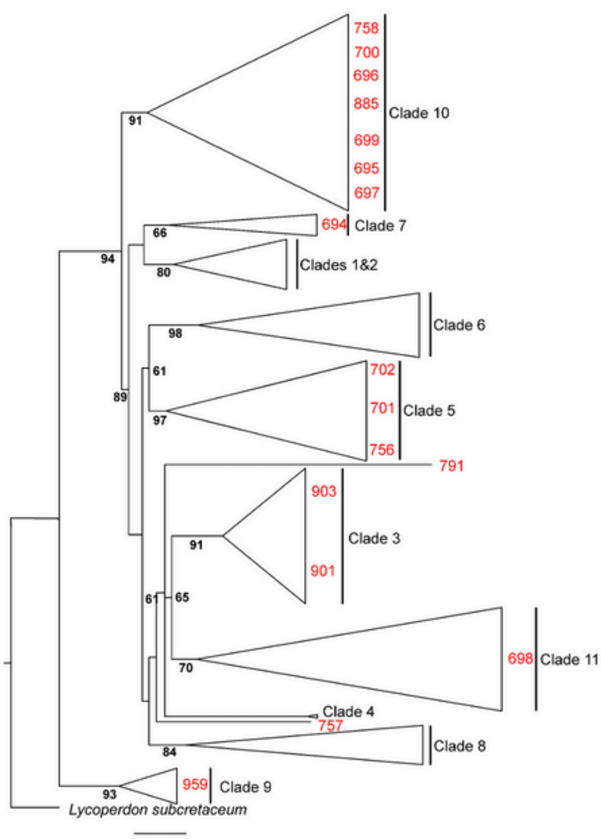

Fig. 1. Maximum likelihood topology inferred for ITS and LSU nuclear sequence matrices combined. Numbers below the branches represent bootstrap support values after 1000 replicates. Red numbers indicate the code of the Mexican *Tulostoma* specimens used in this analysis. We found eleven clades that were collapsed to represent the complete phylogeny.

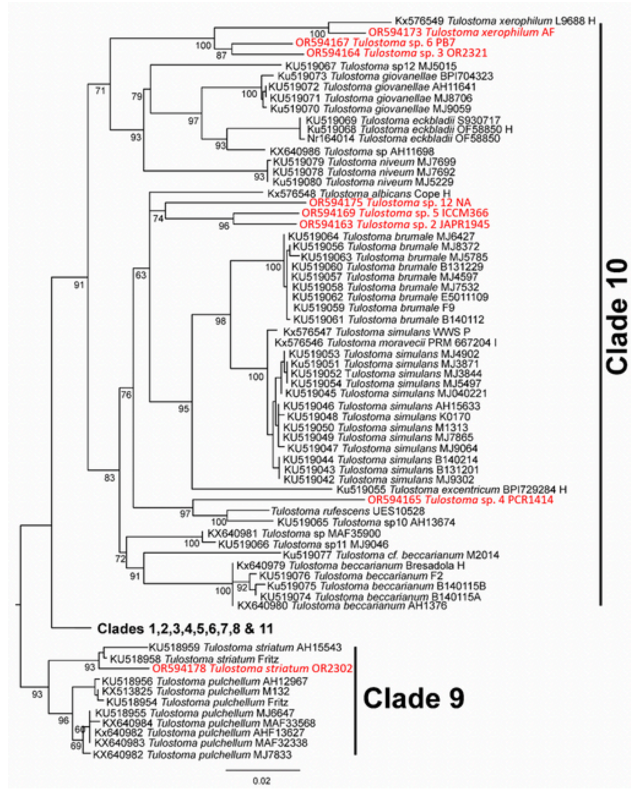

Fig. 2. Partial maximum likelihood topology showing the phylogenetic relationships of the species belonging to clades 9 and 10. Mexican *Tulostoma* specimens are in red. Numbers below the branches represent bootstrap support values after 1000 replicates.

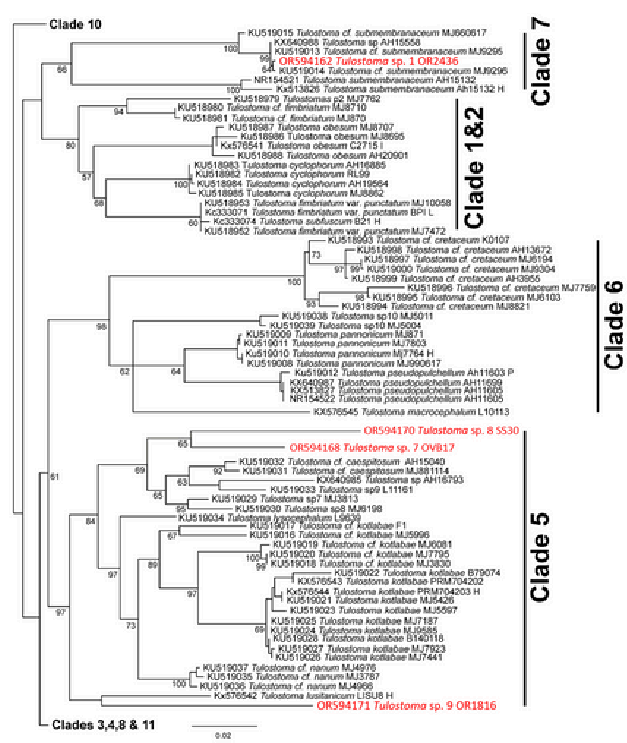

Fig. 3. Partial maximum likelihood topology showing the phylogenetic relationships of the species belonging to clades 1, 2, 5, 6, and 7. Mexican *Tulostoma* specimens are in red. Numbers below the branches represent bootstrap support values after 1000 replicates.

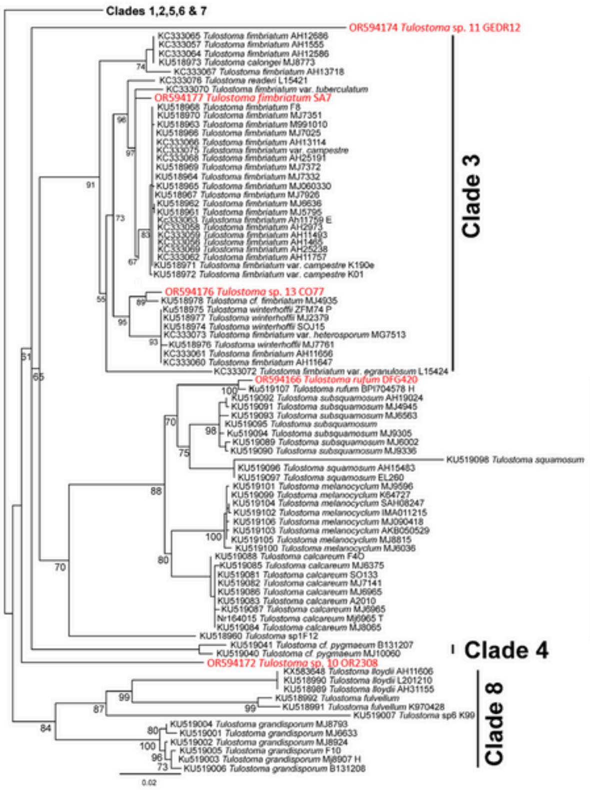

Fig. 4. Partial maximum likelihood topology showing the phylogenetic relationships of the species belonging to clades 3, 4, 8, and 11. Mexican *Tulostoma* specimens are in red. Numbers below the branches represent bootstrap support values after 1000 replicates.

Taxonomy
Descriptions of new records for Mexico

Tulostoma rufum Lloyd, Mycol. Writ. (Cincinnati) (7): 18 (1906). Figure 5.

Spore-sac globose, 10 mm in diameter. **Exoperidium** membranous, redish-brown. **Endoperidium** with tiny scales surface, united forming a reticulum and leaving exposed areas (probably due to detachment), beige-yellowish like straw with redish-brown scales. **Mouth** conspicuous, tubular, 2 mm in diameter, slightly projected, 0.5 mm heigh, with fimbriated border, whitish-yellowish light to grayish. **Peristome**, which is not delimited. **Socket** detached from stem, with a lacerated membrane, dark brown color. **Gleba** whitish. **Stem** subwoody, 35 × 3 mm, cylindrical, little flexuous, at the base a cup-shaped volva, with an irregular margin, attached to the stem, scabrous surface and longitudinal striate, with a woody appearance, dark brown like cinnamon, abundant mycelial cord, difficult to remove.

Spores 6-7.6 × 5.6-6.8 µm and 5.2-5.6 × 4.8-5.2 µm, globose to subglobose, few broadly ellipsoid, Q = 1-1.18, under LM, with large spines, which fuse and form ridges, but do not a complete reticulum, yellowish or green-olive; under SEM the ornamentation appears formed by crests.

Capillitium septate, little branched, 2-6 µm in diameter, tick-walled, 0.5-2.5 µm thickness, slightly flexuous, with or without lumen, some moniliform segments, barely widened at the septum, hyaline to light olive green and yellowish-brown in mass. **Exoperidium** formed by a layer of hyphae, 2.6-5.05 µm diameter, septate, sub-tick walled, 0.39-0.45 µm thickness, hyaline to yellowish in mass.

Habitat: Terrestrial (in soil), on a Stone Wall in an open area, solitary, oak forest.

Material studied: JALISCO, Municipio Zapopan, Comunidad Ecológica Los Guayabos, july 27, 2019, 1653 m s.n.m., *D. Figueroa-García 420*, IBUG.

Observations: The material studied agrees with what was cited by Wright (1987) as Tulostoma rufum. There is no type specimen, so the author considered one of Lloyd´s collections as lectotype based on the original description.

Based on the phylogenetic analysis (Fig. 4) the identification of the material examined here as *Tulostoma rufum* (OR594166) closely related to the lectotype *T. rufum* (KU519107) is supported with a bootstrap value of 100%. *Tulostoma rufum* is recorded for first time from Mexico, a species previously reported of North America, Africa and Europe (Wright, 1987).

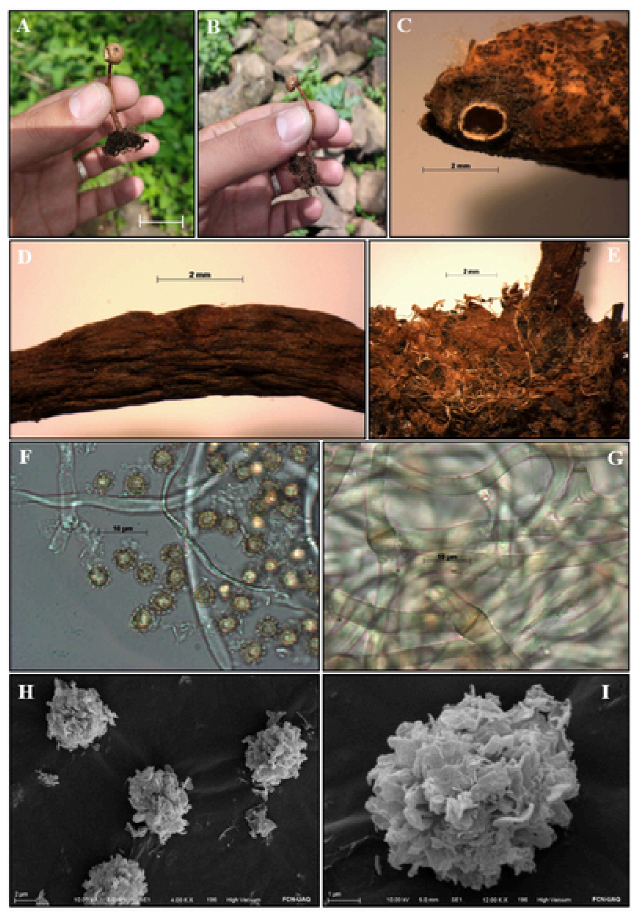

Figure 5. *Tulostoma rufum.* A, B- basidiome ex situ (Bar=13 mm), C- mouth, D- stem striated and scaly, E- base, F- spores (in LM), G- capillitium, H/I- spores (in SEM).

Descriptions of new reports from Jalisco and Zacatecas

Tulostoma fimbriatum Fr., Syst. mycol. (Lundae) 3(1): 43 (1829). Figure 6

Spore-sac globose, 13 mm in diameter. **Exoperidium** difficult to define, whitish-grayish. **Endoperidium** whitish, with remains of sandy substratum particles, finely tomentose surface. **Mouth** subtubular, 3 mm of diameter, projected up to 1 mm heigh, with fimbriated border, concolorous with the surface of the endoperidium. **Peristome** unbounded. **Socket** separated from the stem, with a lacerated membrane margin, light brown-orange color. **Gleba** light yellowish-brown. Stem subwoody, 25-35 × 3-3.5 mm, cylindrical, with a widened base due to the agglomeration of mycelium and sandy particles of the substrate, squamous-fibrillose, brown to light orange-brown in color.

Spores 5.5-6 ×5.5-6 µm, globose to subglobose, Q = 1-1.09, under LM, warty, ornamented by conical warts, that merge or fused to form short and irregular ridges, olive-green; under SEM, the ornamentation appears formed by irregular verrucae, mostly anastomosed, forming ridges of uneven thickness and height with a subreticulum appearance. **Capillitium** septate, with "Y"-shaped ramifications, 4-7 µm in diameter, thick-walled, 1-2 µm thickness, hyaline to yellowish in mass, with lumen, near the septum the hypha thickens and is yellowish-brown or amber in color.

Habitat: buried in ground, gregarious, semi-arid, dominated by cacti.

Material studied: JALISCO, Municipio de San Juan de los Lagos, a un costado de Av. Lázaro Cárdenas, octubre 10, 2017, 1700 m s.n.m., *S. Aguilera* 7, IBUG.

Observations: According to Moreno *et al*., (1995) *Tulostoma fimbriatum* is a species with variable appearance, characterized by having a fimbriate mouth, hyphal exoperidium, squamulose stipe, that is generally dark brown in color, and spores with warts and crested appearing subreticulate with SEM (Moreno *et al*., 1995, 2001).

The phylogenetic analysis carried out (Fig. 4) shows the Mexican material examined (OR594177) related to *Tulostoma fimbriatum* as observed in the major clade, various specimens determined as such species. *Tulostoma fimbriatum* it is one of the species within the genus *Tulostoma*, most widely distributed, cited for México from the states of Baja California (cited as *Tulostoma fimbriatum* var. *campestre*), Chihuahua and Sonora (Moreno *et al*., 1995, 2001; Esqueda *et al*., 2004; Hernandez-Navarro *et al*., 2020).

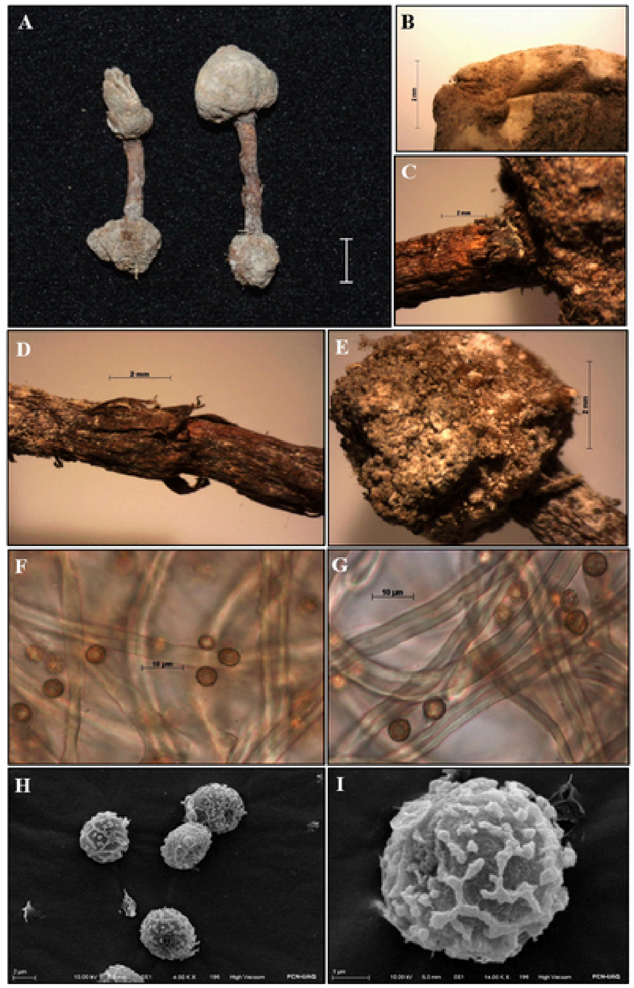

Figure 6. *Tulostoma fimbriatum.* A- basidiomes ex situ (Bar=6 mm), B- mouth, C- socket, D- stem, E- base, F- spores (in LM), G- wall of the capillitium, H/I- spores (in SEM).

Tulostoma striatum G. Cunn., Proc. Linn. Soc. N.S.W. 50(3): 255 (1925). Figure 7

Spore-sac globose, 8.5 mm in diameter, somewhat fragmented by DNA extraction. **Exoperidium** membranous, whitish and covered by sand particles agglutinated. Endoperidium whitish-yellowish, pruinose surface. **Mouth** it could not be observed due to the condition of the specimen, but probably. **Gleba** light yellowish-brown. **Stem** subwoody, 10 × 3 mm, fistulose, longitudinally striate surface, light brown-yellowish, attenuated in the middle portion, with agglomeration of whitish basal mycelium and substrate particles.

Spores 5.5-6 × 5-6 µm, globose to subglobose, Q = 1-1.09, under LM with ribs or striate, with short or straight to spiral, olive-green; under SEM the ribs appear very distinct or conspicuously striated, apiculate. **Capillitium** septate, with lumen, 4.5-11 µm, little branched, thick-walled, wall 1-2 µm thickness, olive-green in color and yellowish in the septum.

Habitat: in dry soil, solitary, grassland vegetation, growing among grass.

Material studied: JALISCO, Municipio Unión de San Antonio, 1 km después del límite con municipio de Lagos de Moreno-Unión de San Antonio, august 30, 2000, 1842 m s.n.m., *O. Rodríguez* 2302, IBUG.

Observations: *Tulostoma striatum* is recognized for presenting a fibrillose-fimbriate mouth, membranous exoperidium, and spores with complete rib-shaped striations that can for spirals. Jeppson *et al*., (2017) mentions that according to molecular data *Tulostoma striatum* is closely related to *T. pulchellum* Sacc., however the species described here is easily distinguished by its striated spores.

Based on the phylogenetic analysis carried out here (Fig. 2), it shows the Mexican material (OR594178) very related to the *Tulostoma striatum* (KU518958, KU518959) with good bootstrap support (93%), and completely separated from the clade *T. pulchellum*.

Tulostoma striatum is widely distributed in America and several continents (Wright, 1987; Altés & Moreno, 1991), for Mexico have been previously registred from the state of Sonora (Wright *et al*., 1972; Esqueda *et al*., 1995). It is cited for the first time from Jalisco.

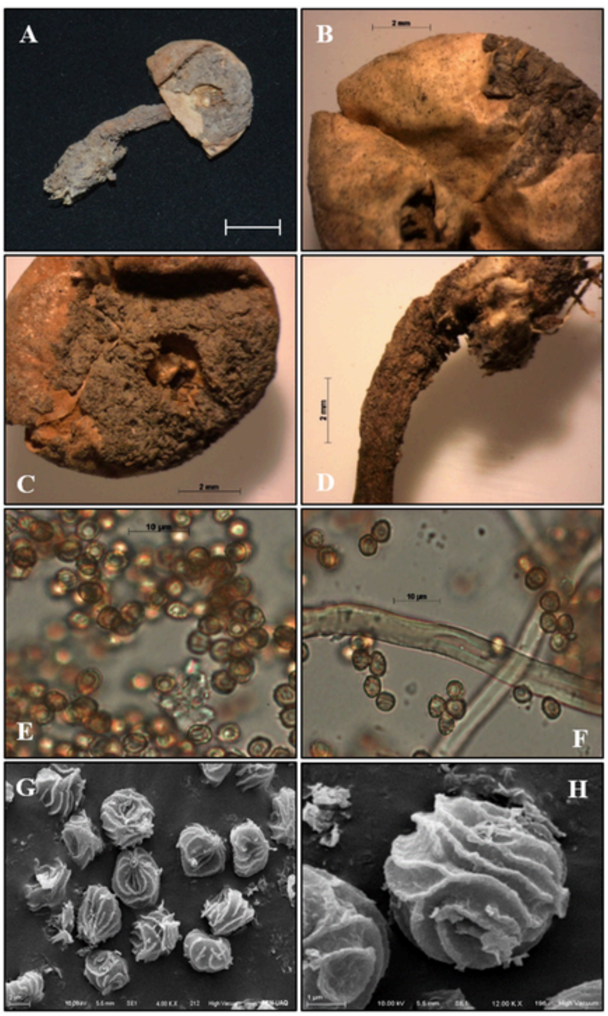

Figure 7. *Tulostoma striatum*. A- basidiome ex situ (Bar=3 mm), B- endoperidium, C- socket, D- stem, E- spores (in LM), F- capillitium, with lumen, G/H- spores (in SEM).

Tulostoma xerophilum Long., Mycologia 38(1): 85 (1946). Figure 8

Spore-sac globose to subglobose, 11-13 mm diameter. **Exoperidium** membranous, brown-yellowish. **Endoperidium** fibrillose-pruinose, in some parts the fibrils are fused to form a network surface, whitish. **Mouth** definite, tubular, 1 mm diameter, projected 0.5-1.7 mm high, with very evident fimbriated border, colored with the surface of the endoperidium. **Peristome** not delimited. **Socket** separated from stem, with a lacerated membrane, light yellowish-brown. **Gleba** light orange-brown. Stem subwoody, 17-20 × 3.5-4 mm, cylindrical, striate-squamous, whitish to light yellow.

Spores 6.5-7.5 × 6-7.5 µm, globose to subglobose, Q = 1-1.15, verrucose cylindrical and conics under LM, warts that fuse to form short, thin ridges but without forming a complete reticulum, orange-yellowish; under SEM, the verrucose to conic that anastamosed forming short ridges, not reticulum. **Capillitium** septate, little branched, 3.5-7 µm diameter, thick-walled, 1-3 µm or more thickness, with visible lumen, with moniliform threads, near at the septum there is thickening and an orange-yellowish color, olive green in mass yellowish. **Exoperidium** formed by hyphae 3-5 µm in diameter, septate, branched hyphae interspersed with remains of the substrate, thin-walled, hyaline.

Habitat: gregarious, without data vegetation.

Material studied: ZACATECAS, without data, august, 2017, A. *Flores s.n*., IBUG.

Observations: *Tulostoma xerophilum* is characterized by its membranous exoperidium, white endoperidium, its tubular mouth and its asperulate spores. A related species is *Tulostoma albicans* V.S. White, a very similar taxon, since both have membranous exoperidium, white endoperidium, and spores that vary from sub-smooth, asperulate to warty, according to what was pointed out by Hernandez-Navarro *et al*. (2020); however, *T. xerophilum* is differentied by the size of the basidiome, more slender, smaller spores and with swollen septa in the capillitium hyaline, this last character not observed in our material examined since it was observed pigmented as described in *T. albicans*.

According to the phylogenetic tree (Fig. 2), the Mexican specimen OR594173 is closely related to the holotype of *Tulostoma xerophilum* (KX576549) included in the same clade with a bootstrap value of 100%. Likewise, the analysis shows the holotype of *Tulostoma albicans* (KX576548) clearly separated from *T. xerophilum* (KX576549), a fact also observed by Jeepson *et al*. (2017).

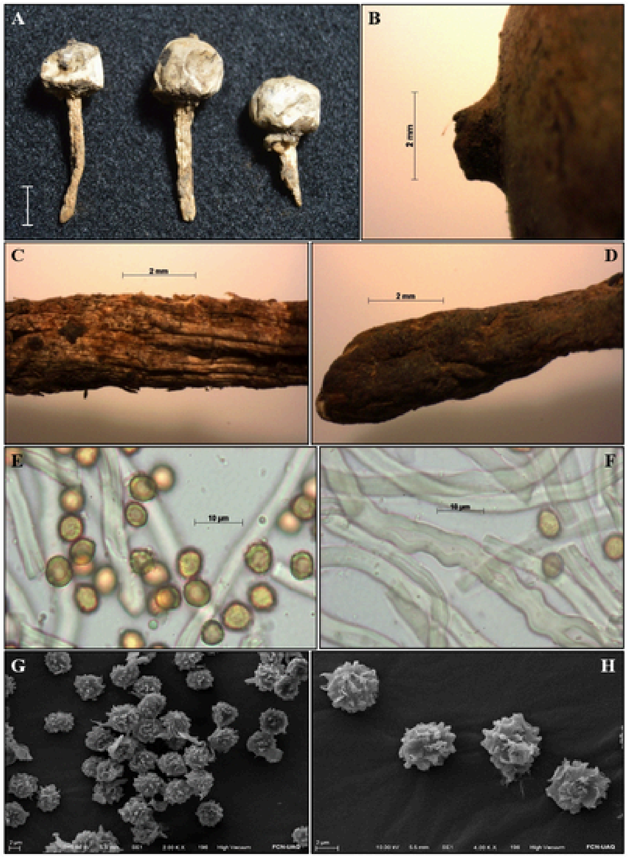

Figure 8. Tulostoma xerophilum. A- basidiomes ex situ (Bar= 6 mm), B- mouth, C- stem, D- base, E- spores (in LM), F- capillitium, G/H- spores (in SEM).

Discussion

In this study, we delineate the species of *Tulostoma* using both morphological and molecular data. We established the phylogenetic placement of our 17 *Tulostoma* specimens, including the 16 species from Mexico (Neotropical region) and one from Spain (Palearctic region). To be consistent with previous studies, we adopted the clade numbering system introduced by Jeppson *et al*. (2017). We identified seven of our *Tulostoma* species within clade 10 (Fig. 1). Notably, *Tulostoma xerophilum* OR594173 from Zacatecas, Mexico was found to be closely related to *T. xerophillum* KX576549 from Arizona, US; this relationship was supported by a 100% bootstrap value. According to Jeppson et al. (2017), *Tulostoma xerophillum* KX576549 serves as the holotype for this species.

Additionally, the specimens corresponding to *Tulostoma* sp. 3 and *Tulostoma* sp. 6 both from Jalisco, Mexico, are sister taxa and form a sister clade with bootstrap support of 87%, suggesting that these two species are new discoveries with the use of other specific primers. These two potential new species from Jalisco, Mexico, are sisters to the clade formed by *Tulostoma xerophilum* OR594173 from Zacatecas and *T. xerophyllum* KX576549 from Arizona, US. This clade is composed of American *Tulostoma* species.

Within the same clade 10, the Mexican specimen identified as *Tulostoma* sp. 2 from Coahuila, Mexico and *Tulostoma* sp. 5 from Jalisco, Mexico, formed a distinct subclade, with a bootstrap support of 96%. Notably, these specimens are clearly separated from the clade grouping *Tulostoma brumale* from clade 10 (Fig. 2). These results suggest the possibility of a potential new species. Additionally, the specimen *Tulostoma* sp. 12 from Nuevo León, Mexico, is identified as the sister taxon to the clade consisting of *Tulostoma* sp. 2 and *Tulostoma* sp. 5 with a bootstrap value of 74%; all these Mexican species are nested into a clade with species of *T. brumale* and *T. simulans* mostly with a Palearctic origin. In concordance with clade 10, the specimen identified as *Tulostoma* sp. 4 which was believed to have microscopic characters like a *T. squamosum* (J.F. Gmel.) Pers. from Querétaro, Mexico, is clearly distinct from the sequence assigned as *T. squamosum* KU519098 from Slovakia, recognized as the species by Jeppson *et al*. (2017) and nested in clade 11 (Fig. 4). Based on these findings, the specimen likely represents a new species, with a support of 97%. Our new taxon (*Tulostoma* sp. 4) is revealed to be the sister species of the clade composed of *Tulostoma rufescens* (MF319226 holotype) from Mexico and KU519065 *Tulostoma* sp. 17 KU519065 (Fig. 2) from Spain.

Clade 9 included to *Tulostoma striatum* OR594178 from Jalisco, Mexico, which was found to be closely related to the clade consisting of *T. striatum* KU518959 from Spain and *T. striatum* KU518958 from Mongolia, with bootstrap support of 93% (Fig. 2). The phylogenetic analysis confirmed that our taxon belongs to *Tulostoma striatum*. The specimens from this clade have a Palearctic and Neotropical origin, indicating the wide geographical distribution of these species.

In clade 7 (Fig. 3), our taxon named *Tulostoma* sp. 1 from Spain was identified as the sister species to *Tulostoma* cf. *submembranaceum* KU519014 also from Spain, with a bootstrap value of 64%. Both collections could potentially correspond to the same new species (*Tulostoma* sp. 15) recognized by Jeppson *et al*. (2017), which does not have a specific name. All the specimens of this clade have a Palearctic origin.

In clade 5 (Fig. 3), the specimens were identified as *Tulostoma* sp.7, sp. 8, and sp. 9 from Jalisco, Mexico. None of these specimens correspond to *Tulostoma squamosum* as they were originally identified without knowing their phylogenetic location. Based on these results, specimens *Tulostoma* sp. 7 and sp. 8 are potentially two new species with a support of 65%. Additionally, a sister species relationship was observed between Tulostoma sp. 9 and the holotype of Tulostoma lusitanicum Calonge & M.G. Almeida from Portugal (Jeppson *et al*., 2017), supported by a 97% bootstrap value.

The specimen *Tulostoma* sp. 11 from Jalisco, Mexico, was found as a sister taxon to clades 3 and 11 (Fig. 4); since these clades have a Palearctic origin, the specimens are clearly distinct from the species included in both clades. This finding suggests that the specimen we analyzed may represent a new species. Similarly, the material identified as *Tulostoma* sp. 10 from Jalisco, Mexico, is the sister species to *Tulostoma* sp. 11 and the clades 3 and 11. This specimen also exhibits characteristics indicating it could be a new species, which is further supported by a bootstrap value of 61%.

In clade 3, the Mexican collection *Tulostoma fimbriatum* OR594177 from Jalisco, Mexico, is closely related to the clade that includes the epitype and lectotype material of the species *T. fimbriatum* (KU518963 from Sweden and KC333075 from US, respectively) as identified by Jeppson *et al*. (2017). Therefore, we can confidently assign this specimen to *Tulostoma fimbriatum*. The inclusion of our sample *Tulostoma fimbriatum* OR594177 indicates that this species is present in the Neotropical, Nearctic, and Palearctic regions. Within clade 3 (Fig. 4), the material identified as *Tulostoma* sp. 13 from Zacatecas, Mexico, was found to be the sister to *Tulostoma* sp. 3 KU518978 from Hungary (*Tulostoma* cf. *fimbriatum* KU518978), a species recognized by Jeppson *et al*. (2017).

In clade 11, the specimen *Tulostoma rufum* OR594166 from Jalisco, Mexico, was closely related to the holotype of *T. rufum* KU519107 from Alabama, US (Fig. 4), with a bootstrap of 100%. Therefore, we conclude that they represent the same species, one distributed in the Nearctic and the other in the Neotropical region. This nested group is also closely related to the clade that groups *Tulostoma subsquamosum* sequences from the Palearctic region, a taxon recognized by Jeppson *et al*. (2017).

The phylogenetic analysis did not support the previous species identification based on morphological traits of most of the 16 Mexican *Tulostoma* specimens. In contrast, our analysis did support the identification of *Tulostoma fimbriatum, T. rufum, T. striatum* and *T. xerophilum*. However, the specimen identified as *Tulostoma* sp. 1 appears to be closely related to *Tulostoma* sp. 15 (KU519014), suggesting that they represent the same species.

Our results using Mexican specimens from the Neotropical region confirm that *Tulostom*a exhibits significant morphological variation, which is still an important factor in species identification. This variability could be especially relevant for pseudocryptic species, in which morphological differences are not easily distinguishable and thus require molecular data to support their identification. The phylogenetic analysis also revealed a substantial number of probable new species and Paleotropical and Paleartic *Tulostoma* species present in the Neotropical region. These findings confirm the high diversity of the *Tulostoma* genus throughout Mexico and potentially the Americas, surpassing initial predictions. Similar conclusions have also been drawn from taxa in Europe and Asia.

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Annex 1.

Sequences of Tulostoma used in this analysis. New rDNA ITS and LSU sequences obtained in this work is in boldface. Lycoperdon subcretaceum was used as outgroup.

Taxa	Country	Voucher	GenBank Accession	References
Tulostoma albicans	USA	Cope Holotype	Kx576548	Jeppson et al. (2017)
Tulostoma beccarianum	Spain	AH1376	KX640980	Jeppson et al. (2017)
Tulostoma beccarianum	Italy	Bresadola Holotype	Kx640979	Jeppson et al. (2017)
Tulostoma beccarianum	Hungary	F2	KU519076	Jeppson et al. (2017)
Tulostoma beccarianum	Slovakia	B140115B	Ku519075	Jeppson et al. (2017)
Tulostoma beccarianum	Slovakia	B140115A	KU519074	Jeppson et al. (2017)
Tulostoma brumale	Norway	E501110	KU519062	Jeppson et al. (2017)
Tulostoma brumale	Sweden	MJ6427	KU519064	Jeppson et al. (2017)
Tulostoma brumale	Sweden	MJ5785	KU519063	Jeppson et al. (2017)
Tulostoma brumale	Czech Republic	B140112	KU519061	Jeppson et al. (2017)
Tulostoma brumale	Czech Republic	B131229	KU519060	Jeppson et al. (2017)
Tulostoma brumale	Hungary	F9	KU519059	Jeppson et al. (2017)
Tulostoma brumale	Slovakia	MJ7532	KU519058	Jeppson et al. (2017)
Tulostoma brumale	Sweden	MJ4597	KU519057	Jeppson et al. (2017)
Tulostoma brumale	France	MJ8372	KU519056	Jeppson et al. (2017)
Tulostoma calcareum	Sweden	Mj6965 Holotype	Nr164015	Jeppson et al. (2017)
Tulostoma calcareum	Hungary	F4O	KU519088	Jeppson et al. (2017)
Tulostoma calcareum	Sweden	MJ6965	KU519087	Jeppson et al. (2017)
Tulostoma calcareum	Sweden	MJ6375	KU519085	Jeppson et al. (2017)
Tulostoma calcareum	Sweden	MJ8065	KU519084	Jeppson et al. (2017)
Tulostoma calcareum	Spain	A2010	KU519083	Jeppson et al. (2017)
Tulostoma calcareum	Sweden	MJ7141	KU519082	Jeppson et al. (2017)
Tulostoma calcareum	Norway	SO133	KU519081	Jeppson et al. (2017)
Tulostoma calcareum	Sweden	Mj6965 Holotype	Ku519086	Jeppson et al. (2017)
Tulostoma calongei	Spain	MJ8773 Holotype	Ku518973	Jeppson et al. (2017)
Tulostoma calongei	Spain	AH13718	Kc333067	Jeppson et al. (2017)
Tulostoma calongei	Spain	AH12686	Kc333065	Jeppson et al. (2017)
Tulostoma calongei	Spain	AH12586	Kc333064	Jeppson et al. (2017)
Tulostoma calongei	Spain	AH1555	Kc333057	Jeppson et al. (2017)
Tulostoma aff. *cretaceum*	Spain	AH3955	Ku518999	Jeppson et al. (2017)
Tulostoma aff. *cretaceum*	Spain	AH13672	Ku518998	Jeppson et al. (2017)
Tulostoma aff. *cretaceum*	Spain	MJ6194	Ku518997	Jeppson et al. (2017)

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Tulostoma aff. *cretaceum*	Spain	MJ9304	Ku519000	Jeppson et al. (2017)
Tulostoma aff. *cretaceum*	Hungary	MJ6103	Ku518995	Jeppson et al. (2017)
Tulostoma aff. *cretaceum*	Hungary	MJ7759	Ku518996	Jeppson et al. (2017)
Tulostoma aff. *cretaceum*	Russia	K0107	Ku518993	Jeppson et al. (2017)
Tulostoma cf. *cretaceum*	Hungary	MJ8821	KU518994	Jeppson et al. (2017)
Tulostoma cyclophorum	Hungary	MJ8862	KU518985	Jeppson et al. (2017)
Tulostoma cyclophorum	Argentina	AH19564	KU518984	Jeppson et al. (2017)
Tulostoma cyclophorum	Spain	AH16885	KU518983	Jeppson et al. (2017)
Tulostoma cyclophorum	Spain	RL99	KU518982	Jeppson et al. (2017)
Tulostoma eckbladii	Norway	Of58850 Holotype	Nr164014	Jeppson et al. (2017)
Tulostoma eckbladii	Norway	S930717	KU519069	Jeppson et al. (2017)
Tulostoma eckbladii	Norway	OF58850 Holotype	Ku519068	Jeppson et al. (2017)
Tulostoma egranulosum	Australia	L15424 Holotype	Kc333072	Jeppson et al. (2017)
Tulostoma excentricum	USA	BPI729284 Holotype	Ku519055	Jeppson et al. (2017)
Tulostoma fimbriatum	Sweden	M991010 Epitype	Ku518963	Jeppson et al. (2017)
Tulostoma fimbriatum	Slovakia	MJ7351	KU518970	Jeppson et al. (2017)
Tulostoma fimbriatum	Slovakia	MJ7372	KU518969	Jeppson et al. (2017)
Tulostoma fimbriatum	Hungary	F8	KU518968	Jeppson et al. (2017)
Tulostoma fimbriatum	Slovakia	MJ7926	KU518967	Jeppson et al. (2017)
Tulostoma fimbriatum	Spain	MJ7025	KU518966	Jeppson et al. (2017)
Tulostoma fimbriatum	Sweden	MJ060330	KU518965	Jeppson et al. (2017)
Tulostoma fimbriatum	Slovakia	MJ7332	KU518964	Jeppson et al. (2017)
Tulostoma fimbriatum	Hungary	MJ6636	KU518962	Jeppson et al. (2017)
Tulostoma fimbriatum	Sweden	MJ5795	KU518961	Jeppson et al. (2017)
Tulostoma fimbriatum	Spain	AH25238	KC333069	Jeppson et al. (2017)
Tulostoma fimbriatum	Spain	AH25191	KC333068	Jeppson et al. (2017)
Tulostoma fimbriatum	Spain	AH13114	KC333066	Jeppson et al. (2017)
Tulostoma fimbriatum	Spain	Ah11759 Epitype	Kc333063	Jeppson et al. (2017)
Tulostoma fimbriatum	Spain	AH11757	KC333062	Jeppson et al. (2017)
Tulostoma fimbriatum	Spain	AH11493	KC333059	Jeppson et al. (2017)
Tulostoma fimbriatum	Spain	AH2973	KC333058	Jeppson et al. (2017)
Tulostoma fimbriatum	Spain	AH1465	KC333056	Jeppson et al. (2017)
Tulostoma fimbriatum	**Mexico**	**SA7**	**OR594177**	**This study**
Tulostoma fimbriatum	Russia	K01	Ku518972	Jeppson et al. (2017)
Tulostoma fimbriatum	Russia	K190e	Ku518971	Jeppson et al. (2017)
Tulostoma fimbriatum	USA	L	Kc333075	Jeppson et al. (2017)
Tulostoma fulvellum	Swiss	ZFM745	KU518992	Jeppson et al. (2017)

Tulostoma fulvellum	Slovakia	K970428	KU518991	Jeppson et al. (2017)
Tulostoma giovanellae	Algeria	BPI704323	Ku519073	Jeppson et al. (2017)
Tulostoma giovanellae	Spain	AH11641	KU519072	Jeppson et al. (2017)
Tulostoma giovanellae	Spain	MJ8706	KU519071	Jeppson et al. (2017)
Tulostoma giovanellae	Spain	MJ9059	Ku519070	Jeppson et al. (2017)
Tulostoma grandisporum	Slovakia	B131208	KU519006	Jeppson et al. (2017)
Tulostoma grandisporum	Hungary	F10	KU519005	Jeppson et al. (2017)
Tulostoma grandisporum	Hungary	MJ8793	KU519004	Jeppson et al. (2017)
Tulostoma grandisporum	Hungary	Mj8907 Holotype	Ku519003	Jeppson et al. (2017)
Tulostoma grandisporum	Hungary	MJ8924	KU519002	Jeppson et al. (2017)
Tulostoma grandisporum	Hungary	MJ6633	KU519001	Jeppson et al. (2017)
Tulostoma kotlabae	Slovakia	PRM704203 Holotype	Kx576544	Jeppson et al. (2017)
Tulostoma kotlabae	Slovakia	PRM704202	KX576543	Jeppson et al. (2017)
Tulostoma kotlabae	Czech Republic	B140118	KU519028	Jeppson et al. (2017)
Tulostoma kotlabae	Slovakia	MJ7923	KU519027	Jeppson et al. (2017)
Tulostoma kotlabae	Denmark	MJ7441	KU519026	Jeppson et al. (2017)
Tulostoma kotlabae	Sweden	MJ7187	KU519025	Jeppson et al. (2017)
Tulostoma kotlabae	France	MJ9585	KU519024	Jeppson et al. (2017)
Tulostoma kotlabae	Sweden	MJ5597	KU519023	Jeppson et al. (2017)
Tulostoma kotlabae	Sweden	B79074	KU519022	Jeppson et al. (2017)
Tulostoma kotlabae	Sweden	MJ5426	KU519021	Jeppson et al. (2017)
Tulostoma lloydii	Spain	AH11606	KX583648	Jeppson et al. (2017)
Tulostoma lloydii	Italy	L201210	KU518990	Jeppson et al. (2017)
Tulostoma lloydii	Spain	AH31155	KU518989	Jeppson et al. (2017)
Tulostoma lusitanicum	Portugal	LISU8 Holotype	Kx576542	Jeppson et al. (2017)
Tulostoma lysocephalum	USA	L9639	KU519034	Jeppson et al. (2017)
Tulostoma macrocephalum	USA	L10113	KX576545	Jeppson et al. (2017)
Tulostoma melanocyclum	Hungary	MJ090418	KU519106	Jeppson et al. (2017)
Tulostoma melanocyclum	Hungary	MJ8815	KU519105	Jeppson et al. (2017)
Tulostoma melanocyclum	Sweden	SAH08247	KU519104	Jeppson et al. (2017)
Tulostoma melanocyclum	Sweden	AKB050529	KU519103	Jeppson et al. (2017)
Tulostoma melanocyclum	Sweden	IMA011215	KU519102	Jeppson et al. (2017)
Tulostoma melanocyclum	France	MJ9596	KU519101	Jeppson et al. (2017)
Tulostoma melanocyclum	Hungary	MJ6036	KU519100	Jeppson et al. (2017)
Tulostoma melanocyclum	Russia	K64727	KU519099	Jeppson et al. (2017)
Tulostoma niveum	Sweden	MJ5229	Ku519080	Jeppson et al. (2017)

Tulostoma niveum	Sweden	MJ7699	KU519079	Jeppson et al. (2017)
Tulostoma niveum	Sweden	MJ7692	KU519078	Jeppson et al. (2017)
Tulostoma obesum	USA	C2715 I	Kx576541	Jeppson et al. (2017)
Tulostoma obesum	Spain	AH20901	KU518988	Jeppson et al. (2017)
Tulostoma obesum	Spain	MJ8707	KU518987	Jeppson et al. (2017)
Tulostoma obesum	Spain	MJ8695	Ku518986	Jeppson et al. (2017)
Tulostoma pannonicum	Hungary	MJ7803	Ku519011	Jeppson et al. (2017)
Tulostoma pannonicum	Hungary	MJ7764 Holotype	Ku519010	Jeppson et al. (2017)
Tulostoma pannonicum	Hungary	MJ871	KU519009	Jeppson et al. (2017)
Tulostoma pannonicum	Hungary	MJ990617	KU519008	Jeppson et al. (2017)
Tulostoma pseudopulchellum	Spain	Ah11603 Paratype	Ku519012	Jeppson et al. (2017)
Tulostoma pseudopulchellum	Spain	AH11605 Holotype	Nr154522	Jeppson et al. (2017)
Tulostoma pseudopulchellum	Spain	AH11699	Kx640987	Jeppson et al. (2017)
Tulostoma pseudopulchellum	Spain	AH11605 Holotype	Kx513827	Jeppson et al. (2017)
Tulostoma pulchellum	Czech Republic	M132 Paratype	Kx513825	Jeppson et al. (2017)
Tulostoma pulchellum	Spain	MAF33568	KX640984	Jeppson et al. (2017)
Tulostoma pulchellum	Spain	MAF32338	Kx640983	Jeppson et al. (2017)
Tulostoma pulchellum	Spain	AHF13627	Kx640982	Jeppson et al. (2017)
Tulostoma pulchellum	Hungary	MJ7833	Ku518957	Jeppson et al. (2017)
Tulostoma pulchellum	Mexico	AH12967	KU518956	Jeppson et al. (2017)
Tulostoma pulchellum	Hungary	MJ6647	Ku518955	Jeppson et al. (2017)
Tulostoma pulchellum	Mongolia	Fritz	KU518954	Jeppson et al. (2017)
Tulostoma punctatum	Slovakia	MJ10058	Ku518953	Jeppson et al. (2017)
Tulostoma punctatum	Slovakia	MJ7472	Ku518952	Jeppson et al. (2017)
Tulostoma punctatum	USA	BPI Lectotype	Kc333071	Jeppson et al. (2017)
Tulostoma punctatum	USA	B21 Holotype	Kc333074	Jeppson et al. (2017)
Tulostoma readeri	Australia	L15421	KC333076	Jeppson et al. (2017)
Tulostoma rufescens	Mexico	UES10528 Holotype	MF319226	Jeppson et al. (2017)
Tulostoma rufum	USA	BPI704578 Lectotype	Ku519107	Jeppson et al. (2017)
Tulostoma rufum	**Mexico**	**DFG420**	**OR594166**	**This study**
Tulostoma simulans	Czech Republic	PRM 667204 Isotype	Kx576546	Jeppson et al. (2017)
Tulostoma simulans	USA	WWS Paratype	Kx576547	Jeppson et al. (2017)
Tulostoma simulans	Hungary	MJ5497	KU519054	Jeppson et al. (2017)
Tulostoma simulans	Hungary	MJ4902	KU519053	Jeppson et al. (2017)
Tulostoma simulans	Hungary	MJ3844	KU519052	Jeppson et al. (2017)
Tulostoma simulans	Sweden	MJ3871	Ku519051	Jeppson et al. (2017)
Tulostoma simulans	Austria	M1313	KU519050	Jeppson et al. (2017)

Tulostoma simulans	Austria	MJ7865	KU519049	Jeppson et al. (2017)
Tulostoma simulans	Russia	K0170	KU519048	Jeppson et al. (2017)
Tulostoma simulans	Spain	MJ9064	KU519047	Jeppson et al. (2017)
Tulostoma simulans	Spain	AH15633	KU519046	Jeppson et al. (2017)
Tulostoma simulans	Hungary	MJ040221	KU519045	Jeppson et al. (2017)
Tulostoma simulans	Czech Republic	B140214	KU519044	Jeppson et al. (2017)
Tulostoma simulans	Czech Republic	B131201	KU519043	Jeppson et al. (2017)
Tulostoma simulans	Spain	MJ9302	KU519042	Jeppson et al. (2017)
Tulostoma sp. 1	Hungary	Mj7762	Ku518979	Jeppson et al. (2017)
Tulostoma sp. 10	Hungary	Mj3813	Ku519029	Jeppson et al. (2017)
Tulostoma sp. 10	Spain	MJ6198	Ku519030	Jeppson et al. (2017)
Tulostoma sp. 11	Spain	Mj881114	Ku519031	Jeppson et al. (2017)
Tulostoma sp. 12	Spain	Ah15040	Ku519032	Jeppson et al. (2017)
Tulostoma sp. 12	Spain	Mj8710	Ku518980	Jeppson et al. (2017)
Tulostoma sp. 12	Italy	Ah16793	Kx640985	Jeppson et al. (2017)
Tulostoma sp. 14	Spain	MJ5004	Ku519039	Jeppson et al. (2017)
Tulostoma sp. 14	Spain	MJ5011	Ku519038	Jeppson et al. (2017)
Tulostoma sp. 15	Hungary	MJ660617	KU519015	Jeppson et al. (2017)
Tulostoma sp. 15	Spain	Mj9296	Ku519014	Jeppson et al. (2017)
Tulostoma sp. 15	Spain	Mi9295	Ku519013	Jeppson et al. (2017)
Tulostoma sp. 15	Spain	Ah15558	Kx640988	Jeppson et al. (2017)
Tulostoma sp. 16	Russia	K99	Ku519007	Jeppson et al. (2017)
Tulostoma sp. 17	Spain	Ah13674	Ku519065	Jeppson et al. (2017)
Tulostoma sp. 18	Hungary	Mj7795	Ku519020	Jeppson et al. (2017)
Tulostoma sp. 18	Spain	MAF35900	Kx640981	Jeppson et al. (2017)
Tulostoma sp. 18	Spain	MJ9046	KU519066	Jeppson et al. (2017)
Tulostoma sp. 19	Cyprus	M2014	Ku519077	Jeppson et al. (2017)
Tulostoma sp. 2	Spain	Mj870	Ku518981	Jeppson et al. (2017)
Tulostoma sp. 20	Spain	MJ5015	Ku519067	Jeppson et al. (2017)
Tulostoma sp. 21	Spain	Ah11698	Kx640986	Jeppson et al. (2017)
Tulostoma sp. 3	Hungary	MJ4935	KU518978	Jeppson et al. (2017)
Tulostoma sp. 4	Hungary	F12	Ku518960	Jeppson et al. (2017)
Tulostoma sp. 5	Slovakia	B131207	KU519041	Jeppson et al. (2017)
Tulostoma sp. 5	Slovakia	MJ10060	Ku519040	Jeppson et al. (2017)
Tulostoma sp. 6	Hungary	Mj5996	Ku519016	Jeppson et al. (2017)
Tulostoma sp. 7	Hungary	F1	Ku519017	Jeppson et al. (2017)

Tulostoma sp. 8	Cyprus	Mj6081	Ku519019	Jeppson et al. (2017)
Tulostoma sp. 8	Hungary	Mj3830	Ku519018	Jeppson et al. (2017)
Tulostoma sp. 9	Hungary	Mj4976	Ku519037	Jeppson et al. (2017)
Tulostoma sp. 9	Hungary	Mj4966	Ku519036	Jeppson et al. (2017)
Tulostoma sp. 9	Hungary	Mj3787	Ku519035	Jeppson et al. (2017)
Tulostoma sp. 9	USA	L11161	Ku519033	Jeppson et al. (2017)
Tulostoma squamosum	Slovakia	Ortova971113	KU519098	Jeppson et al. (2017)
Tulostoma squamosum	France	EL260	KU519097	Jeppson et al. (2017)
Tulostoma squamosum	Spain	AH15483	KU519096	Jeppson et al. (2017)
Tulostoma striatum	Spain	AH15543	KU518959	Jeppson et al. (2017)
Tulostoma striatum	Mongolia	Fritz	KU518958	Jeppson et al. (2017)
Tulostoma striatum	**Mexico**	**OR2302**	**OR594178**	**This study**
Tulostoma submembranaceum	Mexico	Ah15132 Holotype	Nr154521	Jeppson et al. (2017)
Tulostoma submembranaceum	Mexico	Ah15132 Holotype	Kx513826	Jeppson et al. (2017)
Tulostoma subsquamosum	Hungary	MJ4956	KU519095	Jeppson et al. (2017)
Tulostoma subsquamosum	Spain	MJ9305	Ku519094	Jeppson et al. (2017)
Tulostoma subsquamosum	Hungary	MJ6563	KU519093	Jeppson et al. (2017)
Tulostoma subsquamosum	Spain	AH19024	KU519092	Jeppson et al. (2017)
Tulostoma subsquamosum	Hungary	MJ4945	KU519091	Jeppson et al. (2017)
Tulostoma subsquamosum	Slovakia	MJ9336	KU519090	Jeppson et al. (2017)
Tulostoma subsquamosum	Hungary	MJ6002	KU519089	Jeppson et al. (2017)
Tulostoma tuberculatum	Canada	Herb. Long (BPI)	Kc333070	Jeppson et al. (2017)
Tulostoma winterhoffii	Spain	Ah11656	Kc333061	Jeppson et al. (2017)
Tulostoma winterhoffii	Spain	Ah11647	Kc333060	Jeppson et al. (2017)
Tulostoma winterhoffii	The Netherlands	Mg7513	Kc333073	Jeppson et al. (2017)
Tulostoma winterhoffii	Sweden	MJ2379	KU518977	Jeppson et al. (2017)
Tulostoma winterhoffii	Hungary	MJ7761	KU518976	Jeppson et al. (2017)
Tulostoma winterhoffii	Germany	ZFM74 Paratype	Ku518975	Jeppson et al. (2017)
Tulostoma winterhoffii	Denmark	SOJ15	KU518974	Jeppson et al. (2017)
Tulostoma xerophilum	USA	L9688 Holotype	Kx576549	Jeppson et al. (2017)
Tulostoma xerophilum	**Mexico**	**AF**	**OR594173**	**This study**
***Tulostoma* sp. 1**	**Spain**	**OR2436**	**OR594162**	**This study**
***Tulostoma* sp. 2**	**Mexico**	**JAPR1945**	**OR594163**	**This study**
***Tulostoma* sp. 3**	**Mexico**	**OR2321**	**OR594164**	**This study**
***Tulostoma* sp. 4**	**Mexico**	**PCR1414**	**OR594165**	**This study**
***Tulostoma* sp. 5**	**Mexico**	**ICCM366**	**OR594169**	**This study**
***Tulostoma* sp. 6**	**Mexico**	**PB7**	**OR594167**	**This study**

***Tulostoma* sp. 7**	**Mexico**	**OVB17**	**OR594168**	**This study**
***Tulostoma* sp. 8**	**Mexico**	**SS30**	**OR594170**	**This study**
***Tulostoma* sp. 9**	**Mexico**	**OR1816**	**OR594171**	**This study**
***Tulostoma* sp. 10**	**Mexico**	**OR2308**	**OR594172**	**This study**
***Tulostoma* sp. 11**	**Mexico**	**GEDR12**	**OR594174**	**This study**
***Tulostoma* sp. 12**	**Mexico**	**NA**	**OR594175**	**This study**
***Tulostoma* sp. 13**	**Mexico**	**CO77**	**OR594176**	**This study**
Lycoperdon subcretaceum	Sweden	MJ9032	JN572908	Jeppson et al. (2017)