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Systematic review of *Tulostoma* (Agaricomycetes: Agaricales) from MEXU National Fungarium: new records and new species

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Abstract

A taxonomic and phylogenetic study of *Tulostoma* specimens deposited in the MEXU Fungal Collection of the National Herbarium is presented. Eleven collections were morphologically characterized with light microscopy (LM) and Scanning Electron Microscopy (SEM). DNA was extracted with a CTAB 3% protocol. The nrITS (ITS1-5.8S-ITS2), nrLSU (28S), and partial *tef1-α* were amplified and sequenced from both directions. The phylogenetic analysis consisted of Maximum Likelihood and Bayesian Inference Analysis of the ITS+28S, and a concatenated matrix of ITS+28S+*tef1-α*. Both analyses recovered 11 clades. *T. exasperatum, T. rufum,* and *T. simulans* are confirmed species in the Mexican Funga. *T. obscurum* materials correspond with *T. punctatum*, a new record for Mexico. Three new species are described: *T. elvirae, T. evangelinae,* and *T. teophili*. Photographs of specimens and micrographs of light and electron scanning microscopy are provided. Comparison with related taxa is discussed. With the new additions, 56 species are currently known in Mexico.

Key words: Arid zones**,** Barcoding**,** Gasteroid fungi, Gasteromycetes, Tulostomataceae, Stalked puffballs

Introduction

The species of *Tulostoma* Pers. (1794: 86), known as stalked puffballs, are characterized by their gasteroid basidiomes composed of a spore sac easily detached from a hollow stipe, and passive sporal dispersion through an apical ostiole (Persoon 1794, Wright 1987). Currently, around 186 species are known worldwide (Index Fungorum 2025). Although they have a cosmopolitan distribution in almost all terrestrial environments, with the exception of the Antarctic, they are exceptionally diverse and abundant in semiarid and arid environments (Esqueda *et al.* 2004, Finy *et al.* 2023, Hernández-Navarro *et al.* 2015, Jeppson *et al.* 2017, Wright 1987).

In the light of the molecular data, the phylogeny of the genus underwent a leap forward with the contribution of Jeppson *et al.* (2017), who sequenced the nrITS1-5.8S-ITS2 (ITS), D1–D2 from nrLSU (28S), and partial Translation Elongation Factor 1α (*tef1-α*) from several collections, including type specimens. Their findings confirmed that the genus is monophyletic and cryptically diverse. They also confirmed the reliable morphological characteristics, including the type of ostiole, the shape of the peridium, and the size and ornamentation of the basidiospores. As a result, further contributions to our understanding of the genus's diversity emerged from Brazil (Cabral *et al.* 2023, Lima *et al.* 2023), Macedonia (Rusevska *et al.* 2019), Hungary (Finy *et al.* 2023), Pakistan (Hussain *et al.* 2016, Niazi *et al.* 2022), Russia (Vlasenko & Vlasenko 2023), Thailand (Paloi *et al.* 2023), Turkey (Akata *et al.* 2021) and Mexico (Hernández-Navarro *et al.* 2018, Hernández-Navarro & Cappello-García 2024, Rodríguez Alcántar *et al.* 2024). Despite these advances, the true diversity of the genus remains a work in progress, as most sequenced collections are from Europe, hence leading to the need to analyze materials from different biomes and countries.

Mexico has a total area of 1,964,375 km², of which more than 50% is considered drylands, including arid, semiarid, hyperarid, and subhumid areas (SEMARNAT 2003, 2006). The genus *Tulostoma* is one of the most representative gasteroid genera in our country, with fifty-one species reported, while *T. rufescens* Hern.-Nav. & Esqueda (2018: 133), and *T. subreticulatum* Hern.-Nav. & Capello (2024: 653) were described based on morphological and molecular data

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(Hernández-Navarro *et al.* 2018, Hernández-Navarro & Cappello-García 2024). In addition, the fungal collection "Sala *Psilocybe*" of the National Herbarium of Mexico (MEXU) contains over 30,000 fungal specimens, including several notable *Tulostoma* collections. Our primary objectives were to characterize these collections and place them phylogenetically within the genus, based on morphology and DNA sequences from the ITS, 28S, and partial *tef1-α* sequences, thereby contributing to the taxonomy and phylogeny of the genus.

Materials and methods

Morphological Examination

The studied materials are deposited in the macromycetes collection "Sala *Psilocybe*" of the National Herbarium (MEXU) of the Instituto de Biología de la Universidad Nacional Autónoma de México (IBUNAM). The specimens were characterized both macroscopically and microscopically, following the methods described by Wright (1987). The color codes in parentheses are from Kornerup & Wanscher (1978). Microscopic features were obtained by mounting basidiome and gleba fragments in KOH 5%. We measure 30 of each, basidospores including ornamentation, capillitial threads, and septa, using Image Pro Plus 7.0 software (Media Cybernetics 2024). A portion of the gleba was sprinkled on carbon tape and metalized with gold-palladium in a QURUM Q 15 OR Rotary Pumped Coater (Lewes, UK) before being examined in a Hitachi SU 1510 SEM (Hitachi, Japan).

DNA extraction, PCR, and sequencing

Genomic DNA was extracted with a modified CTAB protocol (Doyle & Doyle 1987). The gDNA was quantified in a NanoDrop 2000, and its integrity was verified by visualization on a 1% agarose gel stained with GelRed(™). The gDNA was diluted to 10 ng/µL for PCR use. For the amplification of nuclear ribosomal RNA regions, the full ITS1-5.8S-ITS2 and D1–D2-nrLSU (28S), we used the ITS1F/ITS4B, ITS5/ITS4, and LR0R/LR5 primer pairs (Gardes & Bruns 1993, White *et al.* 1990). PCR reactions were performed using PCR Mix 2× (5'BIO, Mexico) according to the manufacturer's instructions in a volume of 20 µL, with a total of 20 ng of gDNA per reaction. For *tef1-α*, we used the primer pairs EF983F/EF2218R, following the PCR conditions described by Rehner & Buckley (2005). PCR products were then visualized in a 1% agarose gel stained with GelRed(™). Successful amplicons were treated with ExoSAP-IT(™) following the manufacturer's instructions. Clean PCR reactions were sequenced from both ends at the "Laboratorio de Secuenciación Genómica de la Biodiversidad y de la Salud" of IBUNAM, using the same primers in each PCR.

Phylogenetic analyses

The obtained sequences were assembled and curated by inspecting their chromatograms with Geneious Prime® 2024.0.7. The obtained sequences were deposited in GenBank. Reference sequences from *Tulostoma* species were downloaded from the NCBI database from Voucher Specimens from related phylogenetic analysis (Cabral *et al.* 2023, Finy *et al.* 2023, Hernández-Navarro *et al.* 2018, Hernández-Navarro & Cappello-García 2024, Hussain *et al.* 2016, Jeppson *et al.* 2017, Lima *et al.* 2023, Niazi *et al.* 2022, Paloi *et al.* 2023, Rodríguez Alcántar *et al.* 2024, Rusevska *et al.* 2019, Varga *et al.* 2019, Vlasenko & Vlasenko 2023). We included only four sequences of the named materials from Jalisco and Zacatecas published by Rodríguez *et al.* (2024), since all the accession numbers led to partial fragments of 180 bp from the 28S. Two matrices were constructed, one with the ITS+28S sequences, in which we used *Phellorinia longistriatica* R.L. Zhao, M.Q. He & J.Xin Li (2021: 1137), *Dictyocephalos xinjiangensis* R.L. Zhao, M.Q. He and J.Xin Li (2021: 1137), and *Coprinus comatus* (O. F. Mull) Pers. (1797: 62) as outgroups (Li *et al.* 2024). The second matrix was a concatenation of ITS+28S+*tef1-α*, which included only the specimens with all three regions available. We used *Lycoperdon perlatum* Pers. (1796: 4), *L. subperlatum* Chang S. Kim & S.K. Han (2016: 112), *Lepiota alba* (Bres.) Sacc. (1887: 37), *Le. mengei* (Kropp & Castellano) T. Lebel & Vellinga (2012: 528), and *Candolleomyces secotioides* (G. Moreno, Heykoop, Esqueda & Olariaga) D. Wacht. & A. Melzer (2020: 1234) as outgroups (Kim *et al.* 2016, Kropp *et al.* 2012, Lebel & Vellinga 2013, Li *et al.* 2024, Moreno *et al.* 2015, Saccardo 1887). Sequences were aligned using the online version of MAFFT 7, employing the G-INS-i strategy (Katoh *et al.* 2002, 2019, Katoh & Standley 2013). The alignments were reviewed in AliView (Larsson 2014), followed by minor manual adjustments to ensure character homology among the taxa. Each matrix was partitioned; the ITS+28S matrix consisted of four partitions (ITS1, 5.8S, ITS2, and 28S), and the concatenated matrix included three additional partitions for *tef1-α* by codon and one by gene. Phylogenetic inferences were estimated using the Maximum Likelihood Method in IQTree ver. 3 for Mac (Wong *et al.* 2025), and the best model for each partition was selected using ModelFinder (Kalyaanamoorthy *et al.* 2017) with 1,000 ultrafast bootstrap resampling replicates (Minh *et al.* 2013). Bayesian analyses were performed using Mr. Bayes v.3.2.7 (Ronquist *et al.*, 2012). The information block for the matrix included the adapted partition models, two simultaneous runs, four Monte Carlo chains, a temperature set at 0.2, and a sampling of 10 million generations with trees sampled every 1,000 generations. The two simultaneous Bayesian runs continued until the convergence parameters were met as assessed by using Tracer v.1.7.2 (Rambaut *et al.* 2018). The clades are named after the oldest named species of each clade. Accession numbers, name of taxon, voucher, country of origin, and type status are indicated in the label of each sequence specimen in the Phylogenetic trees.

Results

The ITS+28S final matrix consisted of 223 sequences and 1674 positions, of which 918 were conserved and 732 were variable. From these, 147 are singletons, and 584 are informative. As suggested by ModelFinder, the best models were: TPM2u+F+G4 for ITS1, TVM+I+G for 5.8S, TVM+G4 for ITS2, and TIM3+I+G4 for 28S. Both phylogenetic trees from ML and BI analyses recovered the same 11 clades and 81 putative species, including unnamed taxa (Figure 1); however, the deep relationships between the main clades were not entirely supported.

The Laceratum Clade (BS/PP= 100/1) consists of seven species with ostioles that tend to be indefinite to subasteliform, dark disarticulated capillitium, and pigmented smooth basidiospores under LM and SEM: *T. dunense* Finy, Jeppson, L. Albert, Ölvedi, Dima & V. Papp (2023: 158), *T. cretaceum* Long (1944: 321), *T. laceratum* Ehrenb. Ex Fr. (1829: 44)., three undescribed species of *T. aff. cretaceum* 1, 2, and 3, and a new species, *T. evangelinae sp. nov.* (Figure 2) with high support (BS/PP= 100/1). Except for the latter, which presents a submembranous exoperidium, the rest of the species present a hyphal exoperidium (Finy *et al.* 2023, Fries 1829, Long 1944).

The Pseudopulchellum Clade (BS/PP= 94/0.6) consists of six species with definite ostioles and ornamented spores that vary from asperulate to verrucose or sub-reticulate; *T. pseudopulchellum* Moreno, Altés & J.E. Wright (1992: 481), *T. pannonicum* Jeppson, Altés, G. Moreno & E. Larss. (2017: 63), *T. catimbauense* A.A. Lima, Accioly, Baseia & M.P. Martín (2023: 400), *T.* sp. 14, *T. macrocephalum* Long (1944: 337), and a new species, *T. teophili sp. nov.* (Figure 3) with high support (BS/PP= 100/1) (Jeppson *et al.* 2017, Lima *et al.* 2023, Long 1944, Moreno *et al.* 1992). This new species is characterized by its robust spore sac, short and thick stipe, tubular ostiole, membranose exoperidium, and asperulate spores on LM that appear membranose to subreticulate with an incomplete reticulum on SEM.

The Kotlabae Clade (BS/PP= 95/0.94) consists of 14 putative species; *T. kotlabae* Pouzar (1958: 815), and its allies, the undescribed species *T.* sp 6, 7, 8, 9, 10 (here splited in two, 10A and 10B), 11 and 12, *T. lusitanicum* Calonge & M.G. Almeida (2000: 302), *T. morenoi* V.A. Vlasenko & A.V. Vlasenko (2023: 28), an Australian 28S sequence of a specimen labeled as *T. macrosporum* G. Cunn. (1925: 252) from Varga et al (2019), which did not match the Mexican material *aff.* to *T. macrosporum* (Figure 4), and *T. lysocephalum* Long (1944: 325), which varies from the aforementioned by having a fibrillose-fimbriate mouth, while all of the others present circular mouth, hyphal exoperidium, pale colours of the basidiomata, and weakly to coarsely ornamented basidiospores (Calonge & Almeida 1992, Calonge, 2000, Cunningham 1925, Long 1944, Pouzar 1958, Vlasenko & Vlasenko 2023).

The Lloydii Clade (BS/PP= 96/0.8) consists of five species, *T. fulvellum* Bres. (1904: 425), *T. aff. fulvellum*, *T. lloydii* Bres. (1904: 423), *T.* sp 16, and T. *grandisporum* Jeppson, Altés, G. Moreno & E. Larss. (2017: 57) (Jeppson *et al.* 2017, Petri 1904). This is the only clade that does not include any Mexican materials so far.

The Pulchellum Clade (BS/PP= 89/0.88) consists of two species with fimbriated-mammeliform ostiole and membranous exoperidium: *T. pulchellum* Sacc. (1890: 118) and *T. striatum* G. Cunn. (1925: 255). The Mexican materials sequenced from *T. pulchellum* from Baja California Sur, a new record for the state, match the assigned species (Figure 5), as well as the partial 28S sequence of Rodríguez *et al.* (2024) for *T. striatum*. Both species present a mameliform ostiole, smooth endoperidium, and a membranous exoperidium. *T. pulchellum* is characterized by its basidiospores with low, blunt verrucae, which may fuse in irregular patterns. It is a subcosmopolitan species in arid and semiarid zones, particularly in sandy soils with xerophilous vegetation, found in North and South America, Europe, Australia, the Middle East, and New Zealand (Saccardo 1888, Wright 1987). *T. striatum* presents striate basidiospores

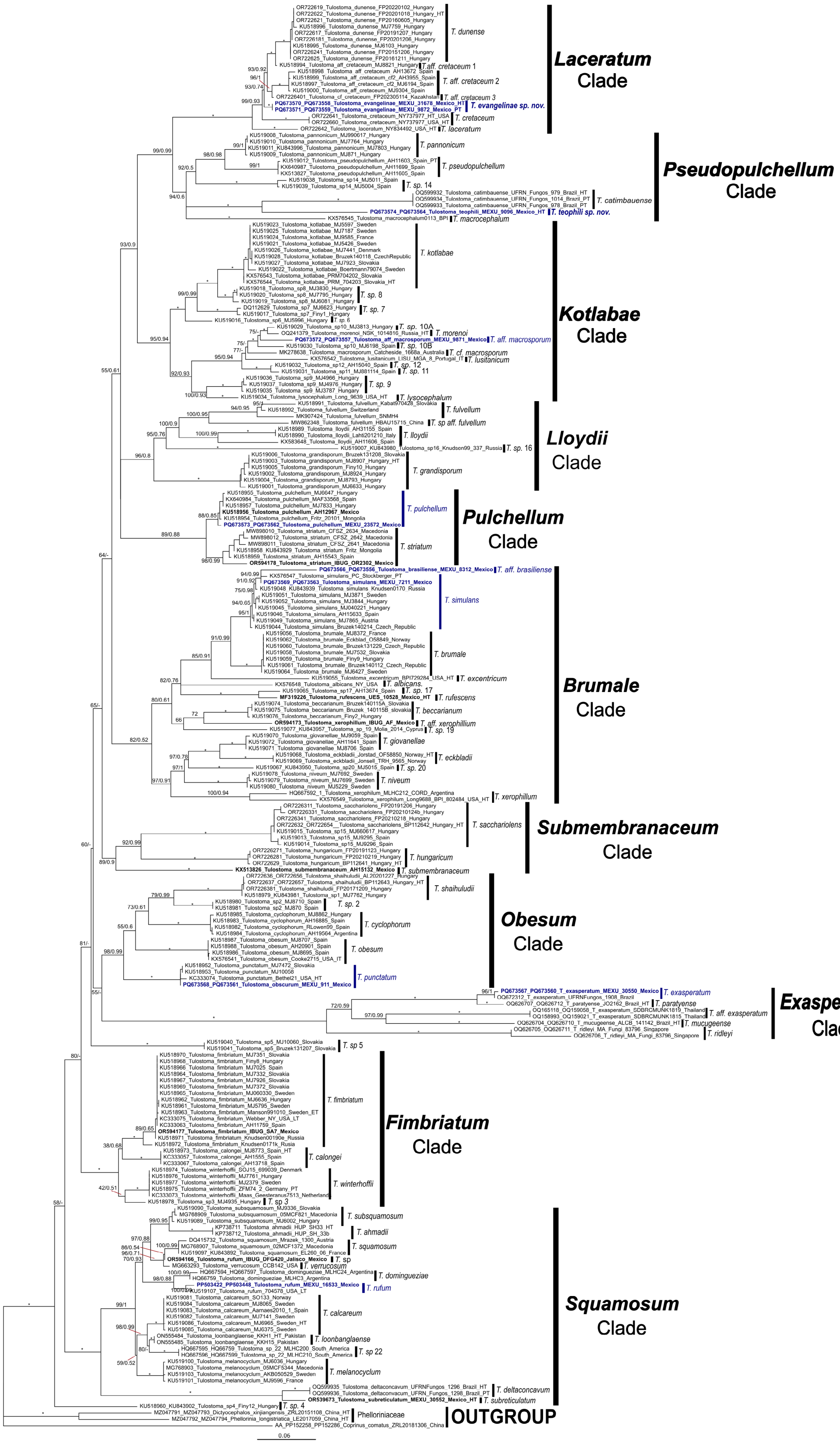


FIGURE 1. Phylogenetic tree of nrITS and nr28S of *Tulostoma.* The Maximum likelihood topology is shown. For each node, the following values are provided: bootstrap (0–100) / and posterior probability (p-value: 0–1). Nodes marked with an asterisk (*) represent a 100/1 support value. The scale bar represents the expected number of nucleotide substitutions per site. The label of each sequence includes NCBI accession number, taxon name, country of origin, and type status: HT = Holotype, LT = Lectotype, PT = Paratype, IT = Isotype. The Mexican specimens are marked in bold black. Newly generated sequences are marked in bold blue.

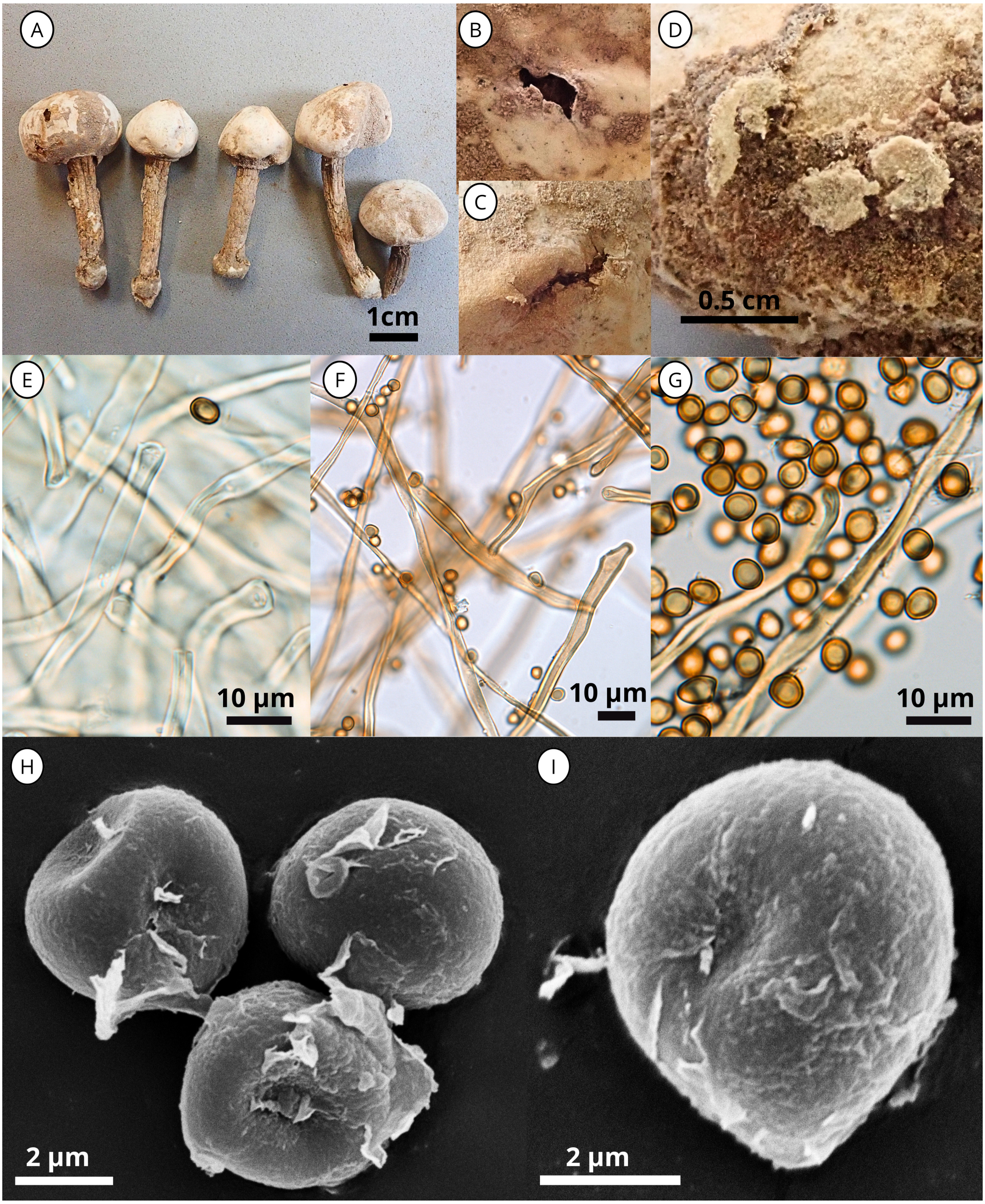


FIGURE 2. *Tulostoma evangelinae* Hern.-Nav. & San Agustín-López (MEXU 31678). A. Basidiomes; B–C. Ostioles; D. Exoperidium; E. Endoperidium hyphae; F–G. Capillitium and basidiospores at LM; H–I. Basidiospores at SEM.

and is also a cosmopolitan in arid and semiarid zones with xerophic vegetation of North and South America, Europe, Australasia, the Middle East, and New Zealand (Cunningham 1925, Saccardo 1888, Wright 1987).

The Brumale Clade (BS/PP= 99/1) consists of 14 species with circular ostiole and asperulate to verrucose basidospores: *T. brumale* Pers. (1794: 86), *T. simulans* Lloyd (1906: 18), *T. excentricum* Long (1944: 332), *T. albicans* White (1901: 428), *T. beccarianum* Bres. (1904: 413), *T. rufescens* Hern.-Nav. & Esqueda (2018: 462), *T. giovanellae* Bres. (1884: 63), *T. eckbladii* Jeppson, Altés, G. Moreno & E. Larss. (2017: 52), *T. niveum* Kers (1978: 411), *T. xerophilum* Long (1946: 85) (including a partial ITS sequence of the type from the USA, and a specimen from Argentina); and the undescribed species *T.* sp 17, 19, and 20 (Hernández-Navarro *et al.* 2018, Kers 1978, Lloyd 1906, Long 1944, 1946, Persoon 1794, Petri 1904, White 1901). The Mexican specimens of *T. simulans* (Figure 6) morphologically and molecularly match the species; however, the specimen of *T. brasiliense* J.E. Wright (1972: 112) from Mexico is also grouped here as a long single branch. The partial 28S sequence of *T. xerophillum* from Jalisco (OR594173) (Rodríguez Alcántar *et al.* 2024, Wright *et al.* 1972) did not match the partial ITS sequence of the holotype from the USA (KX576549). However, it was closer to *T. excentricum,* with low support only from the ML analysis (BS/PP = 61/-). In contrast, in the Bayesian analysis, it was considered a single branch within the genus.

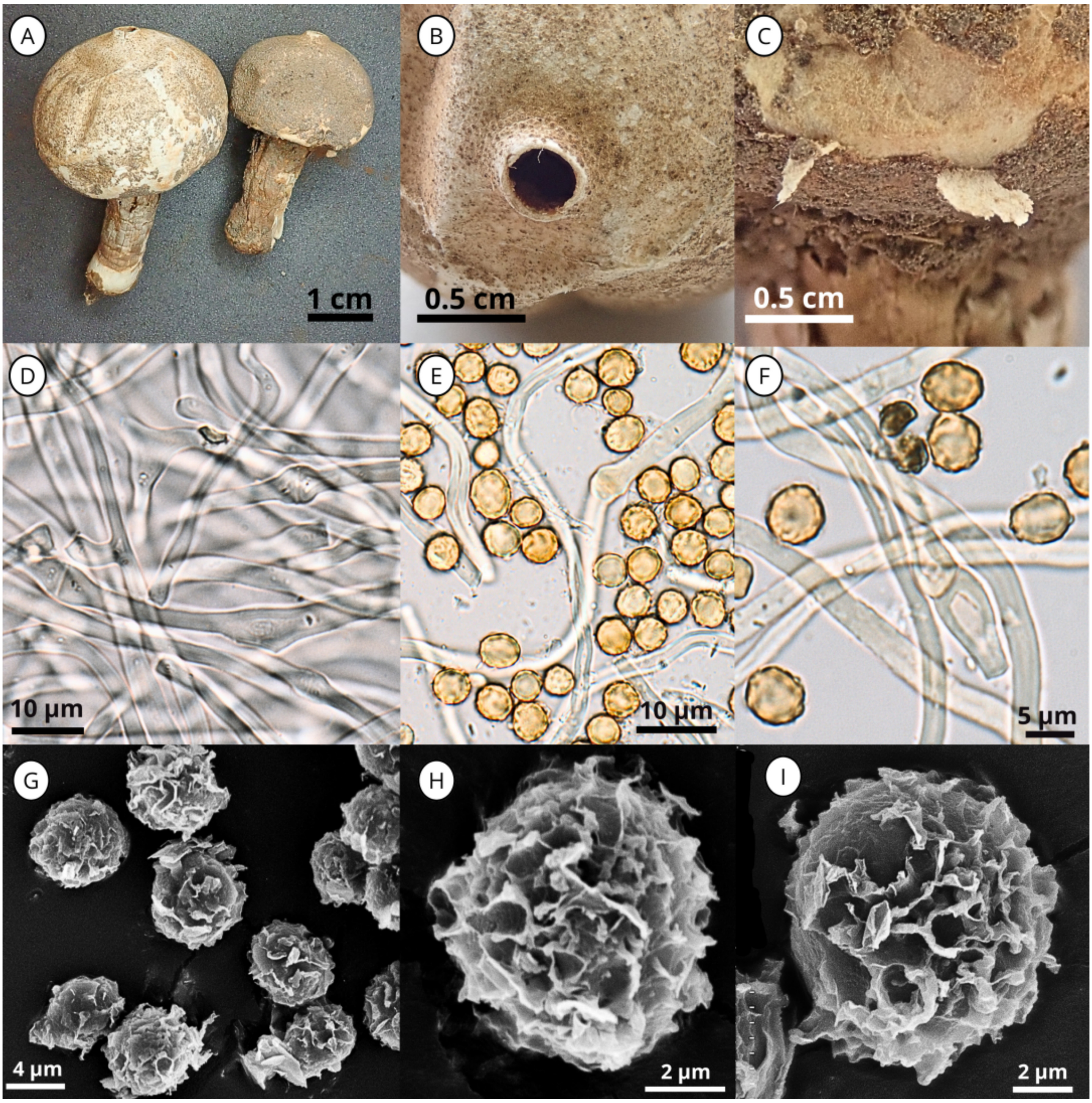


FIGURE 3. *Tulostoma teophili* Hern.-Nav. & San Agustín-López (MEXU 9096). A. Basidiomes; B. Ostiole; C. Exoperidium; D. Endoperidium hyphae; E–F. Capillitium and basidiospores at LM; G–I. Basidiospores at SEM.

The Submembranaceum Clade (BS/PP = 89/0.9) consists of three species, with circular ostiole and asperulate to basidiospores: *T. sacchariolens* Finy, Jeppson, L. Albert, Ölvedi & Dima (2023: 163), *T. hungaricum* Finy, Jeppson, L. Albert, Ölvedi & Dima (2023: 163), and *T. submembranaceum* G. Moreno, C. Ochoa & J.E. Wright (1995: 117) from Mexico (Finy *et al.* 2023, Jeppson *et al.* 2017, Moreno *et al.* 1995).

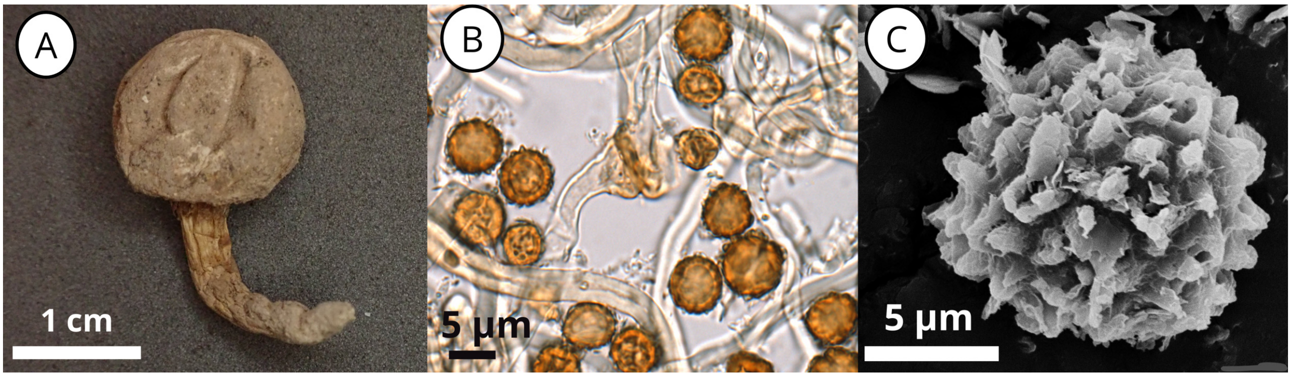


FIGURE 4. *Tulostoma aff. macrosporum* (MEXU 9871). A. Basidiomes; B. Capillitium and basidiospores at LM; C. Basidiospores at SEM.

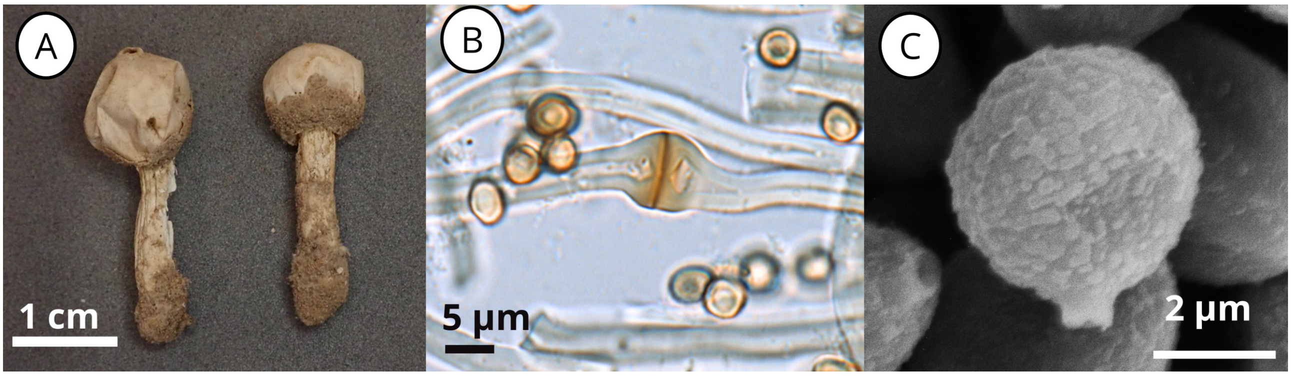


FIGURE 5. *Tulostoma pulchellum* (MEXU 23572) Sacc. A. Basidiomes; B. Capillitium and basidiospores at LM; C. Basidiospores at SEM.

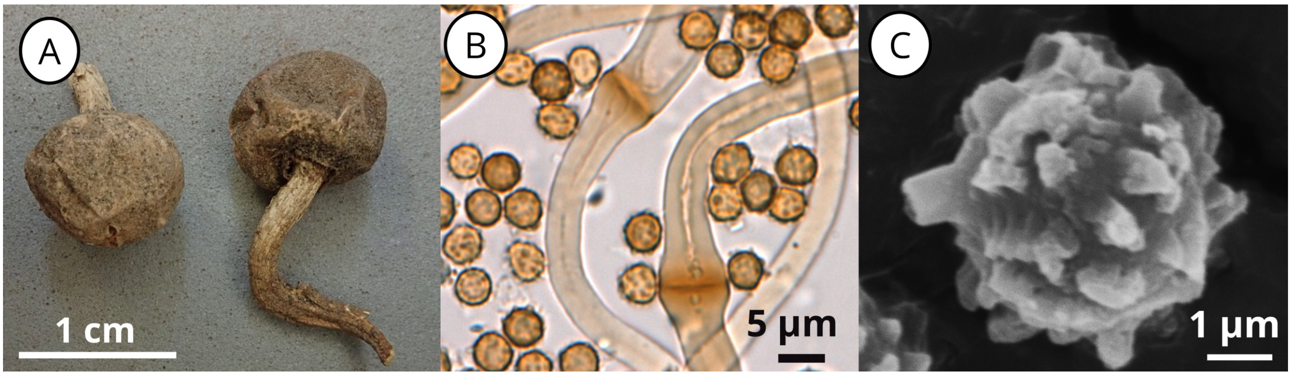
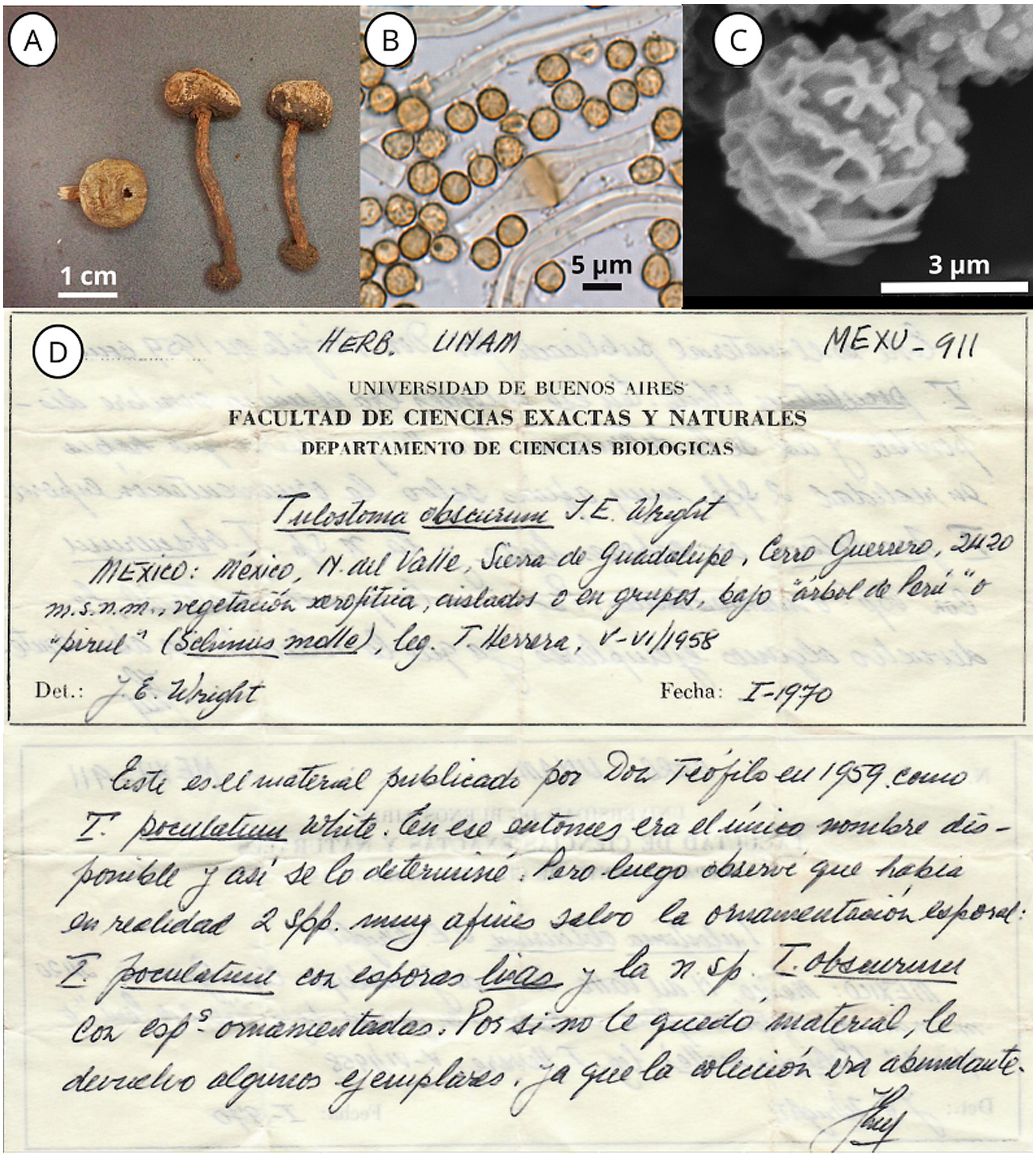


FIGURE 6. *Tulostoma simulans* (MEXU 7211) Lloyd. A. Basidiomes; B. Capillitium and basidiospores at LM; C. Basidiospores at SEM.

The Obesum Clade (BS/PP = 99/1) consists of *T. punctatum* Peck (1986: 419), *T. cyclophorum* Lloyd (1906: 7), *T. obesum* Cooke & Ellis (1878: 82), and *T. shaihuludii* Finy, Jeppson, L. Albert, Ölvedi, D.G. Knapp & Dima (2023: 164). These species present a fibrillose ostiole, hyphal or membranous exoperidium, and smooth to slightly ornamented basidiospores (Cooke & Ellis 1878, Finy *et al.* 2023, Lloyd 1906, Peck 1906). The *T. obscurum* J.E. Wright (1972: 118) materials, published by Wright *et al.* (1972), morphologically and molecularly match *T. punctatum*, a new record for Mexico (Figure 7). This species is characterized by its fibrillose ostiole, hyphal exoperidium, and verrucose basidiospores. Our materials presented basidiospores with fused verrucae (Figure 7C).

The Exasperatum Clade (BS/PP = 100/1), a new clade, consists of five species with a verrucose exoperidium and reticulate basidiospores: *T. exasperatum* Mont (1837: 362), *T. paratyense* T.S. Cabral & B.D.B. Silva (2023: 7), *T.*

ridleyi Massee (1899: 173), *T. mucugeense* (2023: 7), and *T. aff. exasperatum* from Thailand (Cabral *et al.* 2023, Masse 1889, Montagne 1837, Paloi *et al.* 2023). The Mexican materials identified as *T. exasperatum* (Figure 8) match those from Brazil (Cabral *et al.* 2023). This species is characterized by its fibrillose ostiole, verrucose-spiny exoperidium, lignicolous habit, and reticulate basidiospores. The material presented here, sequenced morphologically, aligns with the descriptions of Wright (1987) and Cabral *et al.* (2023). In contrast, the materials from Thailand, grouped as a separate species and here labeled as *T. aff. exasperatum* and differs morphologically (see discussion).



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Tulostoma obscurum J.E. Wright

MEXICO: México, N. del Valle, Sierra de Guadalupe, Cerro Guerrero, 2420 m.s.n.m., vegetación xerofítica, aislados o en grupos, bajo "árbol de Perú" o "pirul" (Schinus molle), leg. T. Herrera, V-VI/1958

Det.: J. E. Wright Fecha: I-1970

Este es el material publicado por Don Teófilo en 1959 como T. poculatum White. En ese entonces era el único nombre disponible y así se lo determiné. Pero luego observé que había en realidad 2 spp. muy afines salvo la ornamentación esporal: T. poculatum con esporas lisas y la n. sp. T. obscurum con esp.s ornamentadas. Por si no le quedó material, le devuelvo algunos ejemplares, ya que la colección era abundante.

FIGURE 7. *Tulostoma punctatum* Peck. (MEXU 911). A. Basidiomes; B. Capillitium and basidiospores at LM; C. Basidiospores at SEM; D. Handwritten note of J. E. Wright with comments on the collection.

The Fimbriatum Clade (BS/PP = 100/1) consists of four species, characterized by a fimbriated ostiole, hyphal exoperidium, and verrucose basidiospores: *T. fimbriatum* Fr. (1829: 43), *T. calongei* Jeppson, Altés, G. Moreno & E. Larss. (2017: 49), *T. winterhoffii* H. Schub. & P. Specht (2013: 421) and *T.* sp 3 (Fries 1829, Jeppson *et al.* 2017, Schubert & Specht 2013). The partial 28S sequence from *T. fimbriatum* by Rodríguez *et al.* (2024) matches the assigned species.

Finally, the Squamosum Clade (BS/PP = 99/1) consists of 12 species with circular ostioles and echinulate to subreticulate basidiospores, in two subclades. In the first subclade: *T. ahmadii* H. Hussain & Khalid (2016: 220), *T. squamosum* (J.F. Gmel.) Pers. (1801: 1), *T. subsquamosum* Long & S. Ahmad (1947: 241), *T. verrucosum* Morgan (1890: 164), *T. domingueziae* Hern. Caff. (2011: 1048), *T. rufum* Lloyd (1906: 18), *T. calcareum* Jeppson, Altés, G.

Moreno & E. Larss (2017: 46), *T. loonbanglaense* Niazi (2022: 3723), *T.* sp. 22, and *T. melanocyclum* Bres (1904: 415). The second subclade includes *T. deltaconcavum* A.A. Lima, Accioly, Baseia & M.P. Martin (2023: 404), and *T. subreticulatum* Hern.-Nav. & Capello (2023: 106) (Hernández Caffot *et al.* 2011; Hernández-Navarro & Cappello-García 2024; Hussain *et al.* 2016; Jeppson *et al.* 2017; Lima *et al.* 2023; Lloyd 1906; Long & Ahmad 1947; Morgan 1890; Niazi *et al.* 2022; Persoon 1801; Petri 1904). The partial 28S sequence of Rodríguez-Alcantar *et al.* (2024), reported as *T. rufum,* does not match the type material and seems to be molecularly and morphologically closer to *T. verrucosum* and *T. squamosum* (see discussion), leading to the discard of the record from Jalisco; however, the MEXU materials sequenced here, identified as *T. rufum* (Figure 9), match the Lectotype, confirming the presence of the species in Puebla, Mexico, with high support (BS/PP = 100/0.99).

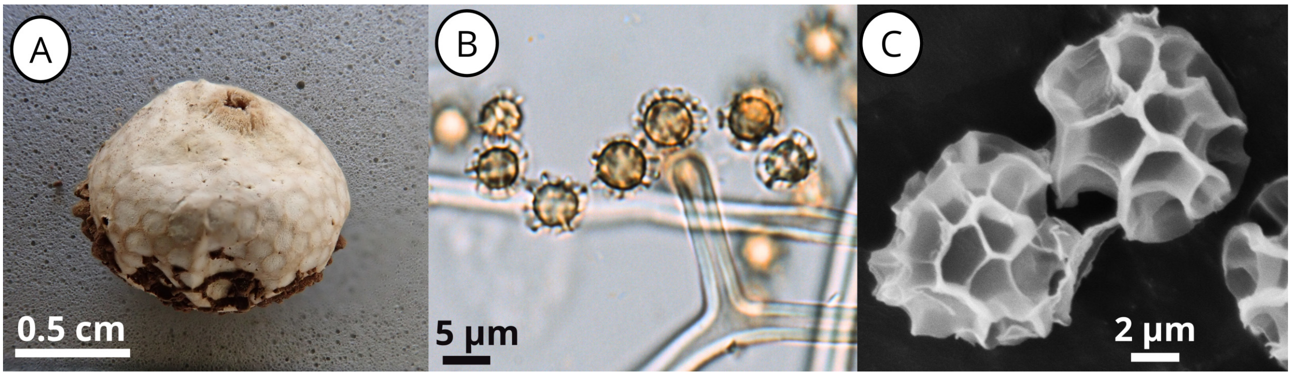


FIGURE 8. *Tulostoma exasperatum* Mont. (MEXU 30550). A. Basidiomes; B. Capillitium and basidiospores at LM; C. Basidiospores at SEM.

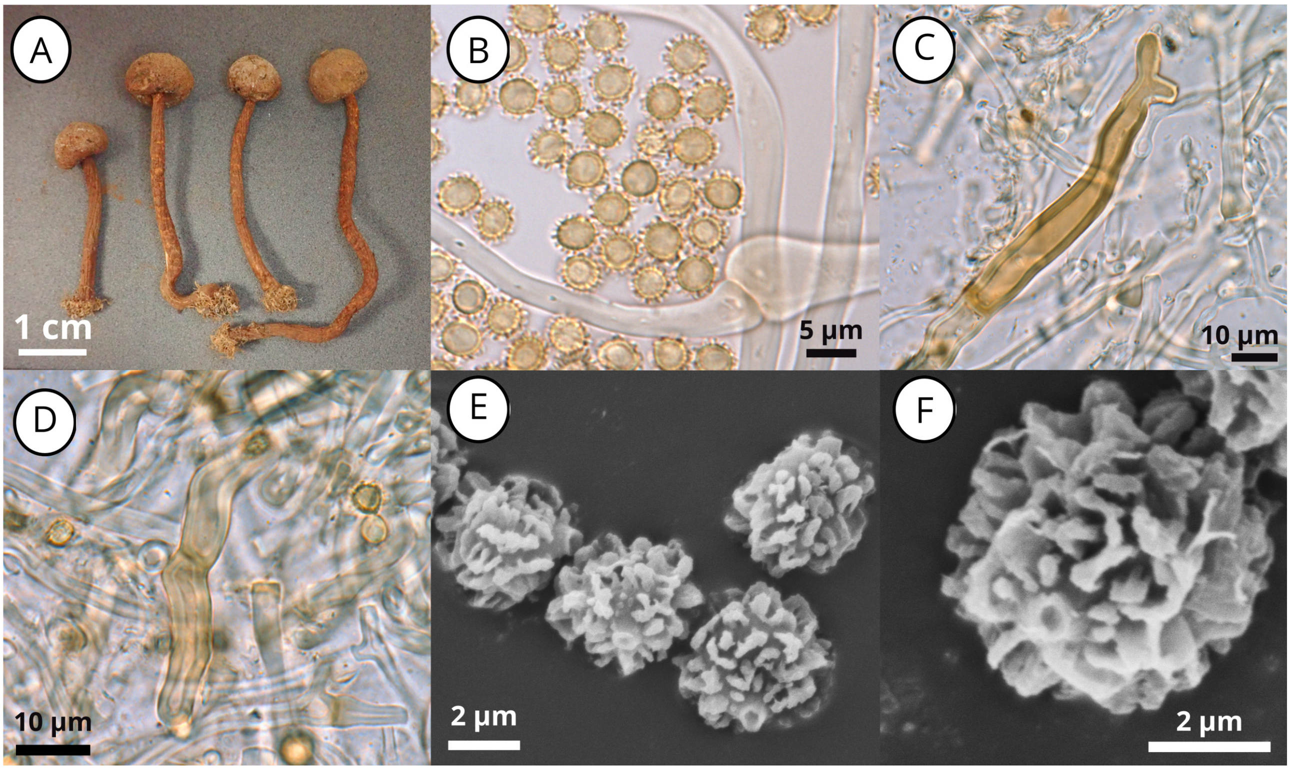


FIGURE 9. *Tulostoma rufum* Lloyd (MEXU 16533). A. Basidiomes; B. Capillitium and basidiospores at LM; C–D. Microsclereids and hyphae of the endoperidium; E–F. Basidiospores at SEM.

On the other hand, the concatenated analysis consisted of 101 sequences and 2,147 positions, of which 1,170 were conserved and 951 were variable. From these, 186 were singletons and 757 were informative. As suggested by ModelFinder, the best models used were: TPM2u+F+G4 for ITS1, TPM2u+F+G4 for 5.8S, TVM+F+G4 for ITS2, TIM3 + F + I + G4 for LSU, and TIM2 + F + G4 for *tef1-α* as gene, and K3Pu+F+G4, TPM2u+G4, and TPM2+FQ+G4 as codons. The topology of the Bayesian analysis is shown (Figure 10). Both trees retrieved the same 11 clades, but only 51 of the species present sequences for all three regions. The two new species, *T. evangelinae* and *T. teophilii*, are also recovered as independent, with high support (BS/PP = 100/1 and 86/0.99, respectively). The material labeled as *T. brasiliense* from Mexico is separated from *T. simulans* and *T. brumale* with high support (BS/PP = 100/1). However, this collection does not entirely align with the concept of *T. brasiliense* as described by Wright (1987) (see discussion). Therefore, now considered a new species, *T. elvirae sp. nov.* (Figure 11).

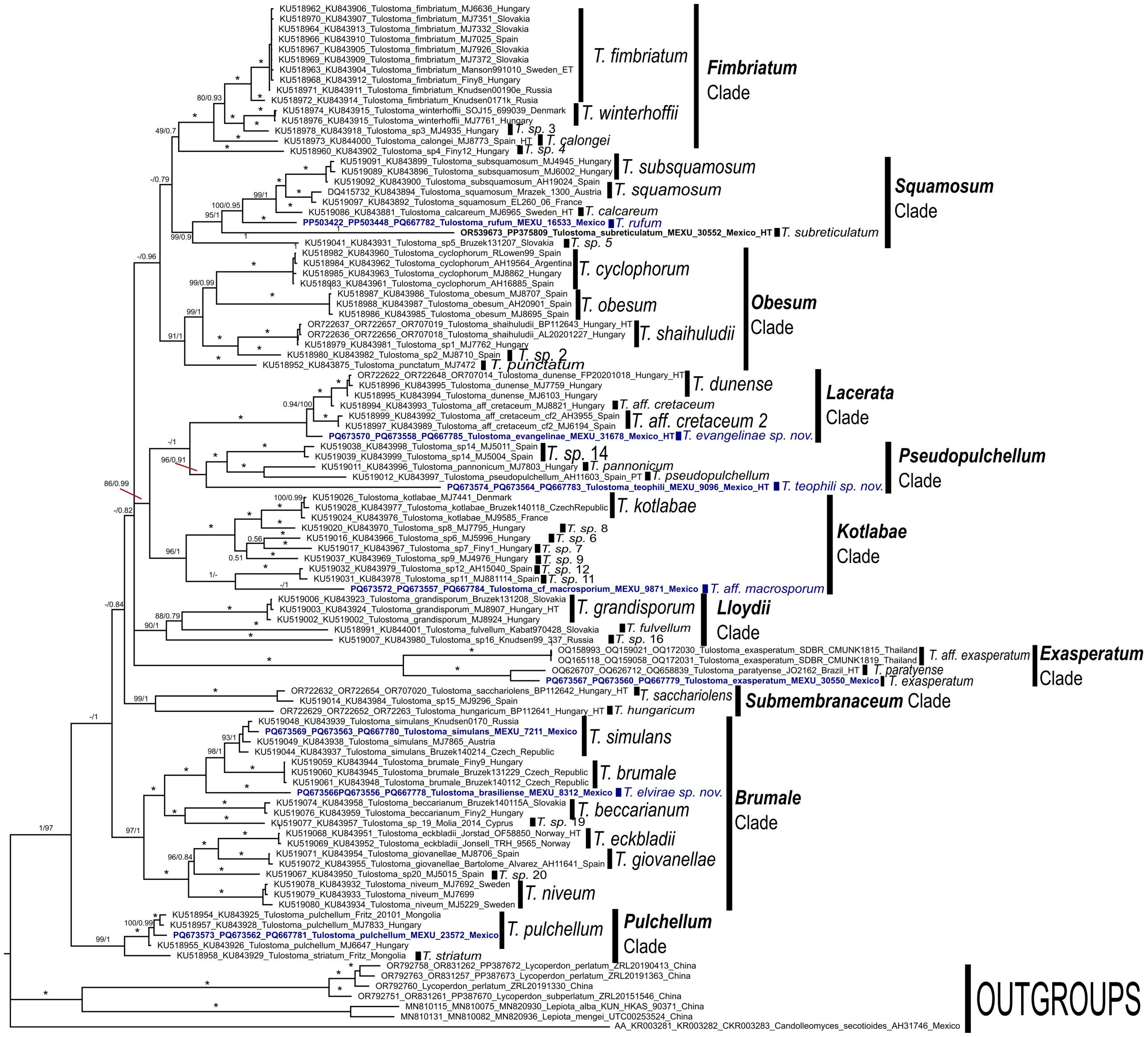


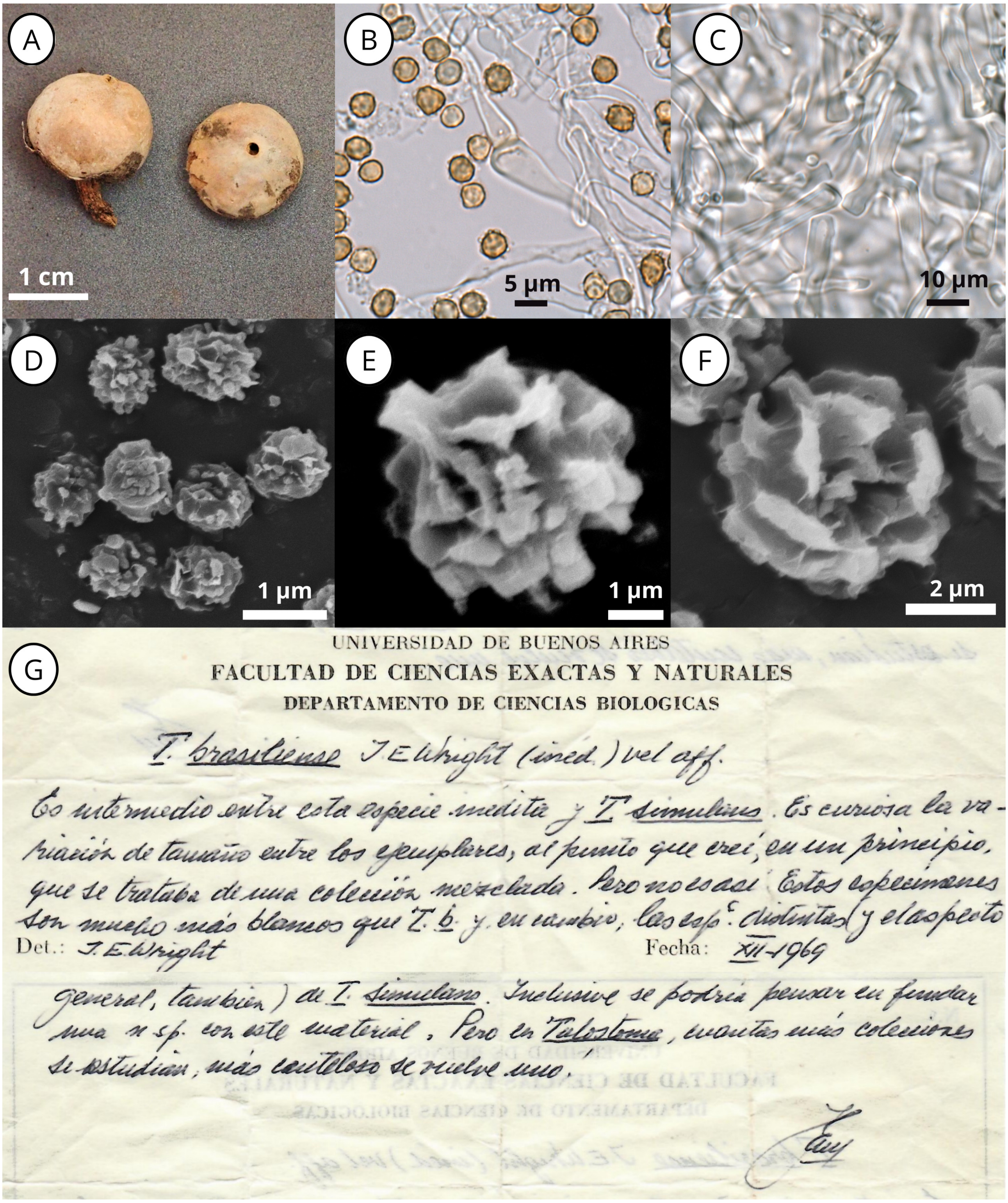
FIGURE 10. Phylogenetic tree of concatenated nrITS, nr28S, and *tef1-α* of *Tulostoma.* The Bayesian topology is shown. For each node, the following values are provided: bootstrap (0–100) / and posterior probability (p-value: 0–1). Nodes marked with an asterisk (*) represent a 100/1 support value. The scale bar represents the expected number of nucleotide substitutions per site. The label of each sequence includes NCBI accession number, taxon name, country of origin, and type status: HT = Holotype, LT = Lectotype, PT = Paratype, IT = Isotype. The Mexican specimens are marked in bold black. Newly generated sequences are marked in bold blue.

Taxonomy

Tulostoma elvirae Hern.-Nav. & San Agustín-López *sp. nov* (Fig. 11)
MykoBank: #860445
GeneBank accession numbers: PQ673566 (ITS), PQ673556 (28S), PQ667778 (*tef1-α*)

Etymology:—dedicated to M. en C. Elvira Aguirre Acosta, curator of the fungal collection in the National Herbarium of the Biology Institute of UNAM (MEXU).

Diagnosis:—Gasteroid basidiomata, stipitated, spore sac 14 mm × 11 mm, tubular ostiole, membranous exoperidium, light orange to pinkish endoperidium, reddish brown stipe, basidiospores 4.7–5.3 µm, irregularly verrucose, yellowish, with bulging verrucae with blunt to conic apex, capillitium hyaline, thin walls, with ribbon-like portions. Septa swollen up to 7 µm.



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DEPARTAMENTO DE CIENCIAS BIOLOGICAS

T. brasiliense J.E.Wright (inéd.) vel aff.

Es intermedio entre esta especie inédita y T. simulans. Es curiosa la variación de tamaño entre los ejemplares, al punto que creí, en un principio, que se trataba de una colección mezclada. Pero no es así. Estos especímenes son mucho más blancos que T. b. y, en cambio, las esp.s distintas (y el aspecto

Det.: J.E.Wright Fecha: XII-1969

general, también) de T. simulans. Inclusive se podría pensar en fundar una n. sp. con este material. Pero en Tulostoma, cuantas más colecciones se estudian, más cauteloso se vuelve uno.

FIGURE 11. *Tulostoma elvirae* Hern.-Nav. & San Agustín-López (MEXU 8321). A. Basidiomes; B. Capillitium and basidiospores at LM; C. Hyphae of the endoperidium. D–F. Basidiospores at SEM; G. Handwritten note of J. E. Wright with comments on the collection.

Type:—MEXICO. Hidalgo: Zimapán, 1773 m asl, 20°44'35"N 99°23'56"W, 12 November 1972, R. Hernández & Y. Vasquez (holotype MEXU 8321).

Macroscopic description:—Spore sac 14 × 11 mm, globose, depressed; ostiole circular, less than 1 mm in diameter, slightly projected. Exoperidium is typically membranous, dark brown (5F4) outside due to adhered particles, orange grey (6B2) inside, falling off in big scales, persisting only around the lower third of the spore sac. Endoperidium payraceous, finally smooth, light orange to pinkish (7C3–6B2). Gleba ferruginous (6B6–6A7), cottony. Socket conspicuous, separated from the stipe. The stipe is broken, and the remaining small portion is striated in parallel, with a woody texture and a slightly reddish-brown color (7B6).

Microscopic description:—Endoperidium conformed by hyaline hyphae 3–5 µm, thick walls up to 2 µm, lumen visible to solid, hyaline swollen septa 5–7 µm. Capillitium 3–6 µm, hyaline, thin walls ≤1 µm, lumen visible, with ribbon-like threads; Septa 4–7 µm, round, swollen, hyaline to yellowish. Basidia not observed. Basidiospores 4.7–5.3 µm, globose, verrucose, some with larger verrucae mixed with smaller ones, yellowish; under SEM, the ornamentation is formed by bulging, blunt to conic verrucae, sometimes fusing in the apex.

Habit:—In sandy soil with vegetation, with *Fouquieria* Kunth (1823: 65) and *Acacia* Mill (1754: 25) *s.l.*

Distribution:—Currently only known from Hidalgo, Mexico.

Notes:—The material sequenced here was initially published by Wright *et al.* (1972) as *T. brasiliense*, along with the holotype specimens from Brazil. Although the Mexican materials were not formally designated as paratypes, they were the only other materials considered in the original description. The collection includes a handwritten note (Figure 11G) by J. E. Wright from 1969, in which he referred to these specimens as "*vel. aff.*" to *T. brasiliense* (ined.) before its formal description and publication in 1972. The note also contains additional text in Spanish, which translates into: "It is intermediate between this unpublished species and *T. simulans*. The variation in size between the specimens is curious, to the point that I believed, at first, that it was a mixed collection. But this is not the case. These specimens are much whiter than *T. b.* (*T. brasiliense*) and, on the other hand, the spores are different (and the general appearance as well) from those of *T. simulans*. One could even think of founding an *n. sp.* with this material, but in *Tulostoma*, the more collections are studied, the more cautious one becomes!" On the other hand, in the world monography, the holotype displays two different sporal ornamentations on SEM, one asperulate and the other verrucose, making this a doubtful species. In addition, Wright (1987) also mentioned that the determination of the Mexican specimens was uncertain, since they were not observed under SEM. *T. brasiliense* was described with pinkish to ochraceous endoperidium, capillitium with thicker septa up to 12 µm, larger basidiospores 6–6.8 (7.6) µm, verrucose with irregular cristae; however, the SEM images of the materials present two different ornamentations, one asperulate (Australian materials, deposited in BPI) and the other coarser with acute conic verrucae (The holotype from Brazil, deposited in BAFC) (Wright 1987). None of them match our materials. Additionally, the type specimens were collected in a tropical forest, while the Mexican specimens were collected in a subtropical thorny-scrub.

Tulostoma evangelinae Hern.-Nav. & San Agustín-López *sp. nov.* (Fig. 2)

MykoBank: #856718

GeneBank accession numbers: holotype: PQ673570 (ITS), PQ673558 (28S), PQ667785 (*tef-1α*); paratype: PQ673571 (ITS), PQ673559 (28S)

Etymology:—dedicated to Dr. Evangelina Pérez-Silva, the first Mexican female mycologist and emeritus researcher from the Biology Institute of UNAM.

Diagnosis:—Gasteroid basidiomata up to 27 mm high, stipitate, 24 × 1.5 mm, stipe with a bulbous structure ending with a rhizomorph stump, spore sac 11 mm × 9 mm, ostiole a simple apical thorn aperture, membranous exoperidium, white to cream-colored endoperidium, basidiospores 4–5 µm, smooth, reddish brown, disarticulated capillitium, with ribbon-like aspect.

Type:—MEXICO. San Luis Potosí, Catorce, San Antonio de Coronados. 23°32'08"N 100°55'55"W, 1928 m asl. 20 December 2015, *E. Flores*, *J. B. Montoya* & *J. D. García* (Holotype MEXU 31678). Paratype (Designated here). Coahuila, Sierra de la Paila, Saucillo. 25°28'12"N 102°59'52"W, 1.103 m asl. 21 October 1975. *G. Vidal Gaona* (MEXU 9872).

Macroscopic description:—Basidiome up to 27 mm high. Spore sac 11 × 9 mm, subglobose, dehiscence through a simple apical aperture. Exoperidium submembranous, grayish-brown outside (6D3), due to adhering soil particles, and whitish inside (6A2), not entirely solid, persisting at the base of the spore sac. The endoperidium is white to cream in color (5A1–6A2), remaining very clean after the exoperidium has been lost. Gleba powdery, dark ferruginous to reddish-brown (6F8–6E8). Socket conspicuous, separated from the stipe, with a membrane. Stipe up to 30 × 6 mm, sand-colored (5A3–5B2) with a conspicuous basal bulb and a stump of a rhizomorph.

Microscopic description:—Endoperidium conformed by hyaline hyphae 3–5 µm diam, thick walls >2 µm, lumen visible to solid, hyaline swollen septa 5–7 µm. Capillitium 5–7 µm diam, reddish brown in the L.M., slightly wavy, lumen visible, with threads with ribbon-like appearance, with solid lumen, easily disjointed at the septa, leaving segments with blunt to rounded ends. Basidia were not observed. Basidiospores 4–5 µm diam, globose, pigmented, entirely smooth under L.M. and SEM.

Habit:—Sandy soils in xerophilic scrub, currently only known in the Chihuahuan Desert.

Distribution:—Chihuahuan Desert: Coahuila and San Luis Potosí, Mexico.

Notes:—*T. evangelinae* is similar to *T. cretaceum* (1944: 36), *T. dunense* (2023: 100), and *T. obesum* Cooke & Ellis (1878: 82). They all share an ostiole that tends to an indefinite aperture, light-colored endoperidium and stipes, with a basal bulb and rhizomorph, powdery reddish-brown gleba, pigmented smooth basidiospores, and capillitium concolorous, disarticulated, with ribbon-like segments (Cooke & Ellis 1878, Finy *et al.* 2023, Long 1944). *T. cretaceum* has been identified as cryptically diverse, with at least three different undescribed species in Europe; however, the holotype is recognized to inhabit the USA (New Mexico), while *T. dunnense* seems to be restricted to the steppes of Hungary. *T. evangelinae* is the only species in the clade that presents a submembranous exoperidium, while the rest show hyphal exoperidia with sand grains incrusted. It also presents a more robust spore sac than *T. dunnense* (up to 27 mm, *vs* 20 mm) and a shorter stem (up to 30 mm *vs* 35–80 mm) (Finy *et al.* 2023).

Tulostoma punctatum Peck, Bull. Torrey bot. Club 23 (10): 419 (1896) (Fig. 7)
GeneBank accession numbers: PQ673568 (ITS), PQ673561 (LSU)

Macroscopic description:—Complete basidiome up to 50 mm high. Spore sac 11 × 9 mm, subglobose. Ostiole fibrillose, almost flat, less than 1 mm in diameter, not delimited. Exoperidium hyphal, mixed with sand grains and persistent at the base of the spore sac. Endoperidium grayish (5B1–5C1) with sand particles. Gleba dark-ferruginous, powdery to slightly cottony. Stipe up to 45 mm, grayish brown (6E4–6E3), striated along its length.

Microscopic description:—Capillitium 5–8 µm, hyaline, some portions ribbon-like, lumen visible. Septa 6–10 µm, swollen, slightly pigmented. Basidia were not observed. Basidiospores 4.7–5.1 µm, yellowish, subglobose, warty. Under SEM, the ornamentation is formed by verrucae, ranging from isolated to fused in anastomosed processes.

Habit:—Sandy soils, xerophilic vegetation, under *Schinus molle* and *Opuntia* sp.

Distribution:—North America (USA, Mexico) and Eurasia (Jeppson *et al.* 2017, Wright, 1987).

Material examined:—MEXICO: CDMX, Sierra de Guadalupe, Cerro Guerrero, 2420 m asl, 19°29'52"N 99°06'22"W, 23 June 1956, *T. Herrera* (MEXU 911). Nuevo León: Doctor Arroyo, 1700 m asl, 23°40'51"N 100°12'05"W, 1 November 1972, *F. González-Medrano s/n* (MEXU 2363).

Notes:—The collection includes a handwritten note (Figure 7G) by J. E. Wright from 1970, which contains additional text in Spanish that translates to: "This material was published by Dr. Teófilo (Herrera 1959) as *T. poculatum* White (1901: 28). At that time, it was the only name available, and that's how I determined it for him. But then I observed that there were actually two spp very similar except for the spore ornamentation: *T. poculatum* with smooth spores and the *n. sp. T. obscurum* with ornamented spores. In case you don't have any material left, I give you back some specimens, since the collection is abundant". Despite not being designated formally as a paratype, this collection was part of the original description of *T. obscurum* (Wright *et al.* 1972), along with other materials from Mexico deposited in the ENCB herbarium. *T. punctatum* is similar to *T. fimbriatum*, which also presents hyphal exoperidium, fibrillose ostiole, and verrucose basidiospores, but presents smaller basidiomes. Jeppson *et al.* (2017) pointed out that *T. punctatum* differs by having basidiospores with independent verrucae, in contrast to *T. fimbriatum*, which presents fused verrucae. Our materials presented fused verrucae.

***Tulostoma rufum* Lloyd**, Mycol. Writ. (Cincinnati) (7): 18 (1906) (Fig. 9)
GeneBank accession numbers: PP503422 (ITS), PP503448 (LSU) PQ667782 (*tef1-α*)

Macroscopic description:—Basidiomata up to 55 mm high. Spore sac subglobose, 5–9 mm diam, 4–6 mm high; mouth circular to elliptical, less than 1 mm in diameter, and in projection; exoperidium hyphal, mixed with sand grains, persistent in the lower third of the spore sac. Endoperidium initially light reddish brown (6C5), whitish to cream (6B2) with tempering, with a darker peristome. Gleba light ferruginous (6B6–6A7), pulverulent to cottony. Socket conspicuous, separated from the stipe. Stipe up to 60 × 2.5 mm, sub-woody, reddish brown (8D6), striated in a spiral pattern, with a conspicuous basal bulb with hyphae strongly mixed with grains of sand and debris as rhizomorph.

Microscopic description:—Endoperidium is formed by short and wavy hyaline hyphae, 3–6 µm in diameter, septated, and slightly swollen up to 10 µm at the yellowish septa. Abundant microsclereids up to 10–25 × 7–11 µm, pigmented, with thick, darker cell walls up to 4 µm, one-celled to septated, rounded to clavate tips. Capillitium hyaline to slightly yellowish, 4–7 µm, with a visible lumen. Septa hyaline to yellowish, swollen 5–8 µm to abruptly swollen up to 12 µm. Basidia were not observed. Basidiospores 5–7 µm, globose to subglobose, yellowish, with echinulate ornamentation consisting of conspicuous and independent spines; under SEM, the ornamentation is formed by coalescent and independent spines.

Habit:—soil with abundant organic matter in a *Liquidambar* L. (1753: 999), *Quercus* L. (1753: 994), and *Cyathea* Sm. (1793: 416) mixed forest.

Distribution:—USA (Lectotype), South Africa, Belgium, France (Wright, 1987), Russia (Gorbunova, I. A. & Rebriev, Y. A. 2016), Mexico (present study).

Material examined:—MEXICO. Puebla: Apulco. 1507 m asl, 19° 54'31.3" N, 97° 36' 27.9" W, 13 September 1980. *E. Pérez-Silva* (MEXU 16533)

Notes:—*Tulostoma rufum* is a distinct species characterized by its medium-sized basidiomes, tubular stoma, hyphal exoperidium, reddish-brown stipe and endoperidium, abundant microsclereids, and echinulate basidiospores with independent spines. In the world monograph (Wright 1987), the description of *T. rufum* did not mention the presence of microsclereids in the peridium; however, these were illustrated in Figure 132, though measures and descriptions were not provided. This species was classified in the subgenus *Tulostoma*, series Tubulares, Sectio Hyphales, which includes 34 other species. Most of them do not resemble *T. rufum*, especially in the microscopic characteristics. A similar species in such Sectio is *T. floridanum* (1906: 7), which shares the hyphal exoperidium, tubular stoma, reddish stipe, and echinulate basidiospores. However, *T. floridanum* lacks mycosclereids and presents a grayish endoperidium with slightly smaller basidiospores (4.6–6.4 µm) (Esqueda *et al.* 2004, Lloyd 1906). Another very similar species is *T. melanocyclum*, which also presents a circular ostiole with a dark peristome, hyphal exoperidium, and spiny basidiospores; however, it presents a white to cinereous endoperidium and lacks microsclereids (Esqueda *et al.* 2004; Wright 1987). Wright (1987) mentioned that this species could be considered a small variety of *T. squamosum*. However, the latter presents a verrucose exoperidium conformed by sphaerocysts and no mycosclereids. Another similar species in the Sectio is *T. subsquamosum*, which also presents a hyphal exoperidium with scattered sphaerocysts and no mycosclereids. Additionally, *T. subsquamosum* has smaller subreticulate basidiospores (4.6–6.4 µm) (Calonge *et al.* 2007, Long & Ahmad 1947, Persoon 1801).

Tulostoma teophili Hern.-Nav. & San Agustín-López *sp. nov.* (Fig. 3)
MykoBank: #856719
GeneBank accession numbers: PQ673574 (ITS), PQ673564 (28S), PQ667783 (*tef1*-α)

Etymology:—dedicated to Dr. Teófilo Herrera Suárez, the first Mexican mycologist devoted to the study of gasteroid macrofungi, and researcher at the Biology Institute of UNAM.

Diagnosis:—Gasteroid basidiomata up to 31 mm high, stipitate, 20 × 5 mm, stipe with a bulbous structure ending with a rhizomorph stump, spore sac up to 17 mm diam × 12 mm height, circular ostiole, membranous exoperidium, cream-colored endoperidium, asperulate to irregularly verrucose basidiospores at LM; membranous, subreticulate at SEM. Hyaline capillitium with swollen septa.

Type:—MEXICO. Chiapas: Road from Tapitula to Rayón, 1.138 m asl, 17°13’08.7”N 93°00’43.8”W, 13 August 1972, *T. Herrera & J. M. Trappe* (holotype MEXU 9096).

Macroscopic description:—Basidiome up to 31 mm high. Spore sac up to 17 × 12 mm, globose, depressed; ostiole tubular, 1 mm in diameter and in projection. Exoperidium membranous, grayish brown outside (5E3–5F4) and whitish to light beige inside (5A2–6A2), persisting in patches. Endoperidium light sand-colored (5A2–6A2). Gleba ferruginous, powdery to slightly cottony. Stipe up to 20 × 5 mm, with a woody texture and a light, sand-colored (5A2–5B2) hue. The end of the stipe widens somewhat as it approaches the base.

Microscopic description:—Endoperidium conformed by hyaline hyphae 3–5 µm, thick walls up to 2 µm, lumen visible to solid, hyaline swollen septa 5–7 µm. Capillitium 3–4 µm, slightly branched, yellowish to hyaline, lumen visible. Septa 4–6 µm, swollen, hyaline to yellowish. Basidia not observed. Basidiospores 7–8 µm, asperulate at LM, under SEM, the ornamentation is formed by irregular membranes resembling an incomplete reticulum.

Habit:—Sandy soil.

Distribution:—Currently only known from Chiapas, Mexico.

Notes:—This material was collected by Dr. Teófilo Herrera and Dr. James Martin Trappe in 1972 and has never been published. This is a distinct species due to the combination of characters: a stout spore sac with a short stipe, a membranous exoperidium, a circular ostiole, and asperulate-reticulate basidiospores. *T. teophili* could be mistaken with *T. macrocephalum*. Still, the latter presents a longer stem (up to 90 × 8 mm) and smaller basidiospores (5.4–8.6 µm), characterized by low verrucae and the absence of membranes or subreticulum. Phylogenetically, *T. teophili* is related to *T. catimbuense*, another species with subreticulated basidiospores described from Brazil. However, the latter presents a hyphal exoperidium, and the basidiospores present a spiny, coarse subreticulum (Lima *et al.* 2023).

Discussion

The materials identified as *T. exasperatum*, *T. pulchellum*, *T. punctatum*, *T. rufum*, and *T. simulans* match morphologically and molecularly with the species concept of each. However, specific points in each species require discussion.

Tulostoma exasperatum s.l. is a pantropical species easily recognizable due to its lignicolous habit or soils with abundant organic matter, as well as for its reddish brown, warty-spiny exoperidium, fibrillose ostiole, and winged-reticulate basidiospores. Wright (1987) classified *T. exasperatum* within the subgenus *Tulostoma*, series *Fimbriata*, and section *Exasperata*, of which it is the type species. The diagnosis consists of a warty exoperidium formed by short and twisted hyphae, and a fibrillose or fimbriated ostiole. This section also contained *T. ridleyi* and *T. transvaalii* Lloyd (1920: 1047). The former is distinguished by its robust spore sacs with a stipe up to 120 mm, larger basidiospores (6.4–8.1 µm), and a distribution limited to Southeast Asia (Wright 1987). The latter, on the other hand, has robust spore sacs measuring up to 22 mm, basidiospores of similar size, but with non-membranous reticulation, and exhibits a terrestrial habit, developing in clay soils only in South Africa (Lloyd 1920, Wright 1983, 1987). *T. exasperatum s.l.* is considered pantropical, since it presents numerous reports, from Africa: Congo, Ivory Coast, Ruanda, Zaire (Wright 1987). America: Argentina (Spegazzini 1927), Brazil (Baseia & Galvão 2002, Baseia & Milanez 2002, Bononi *et al.* 1984, Cabral *et al.* 2023, Rick 1961), Cuba, Hawaii Islands (Long 1947), Mexico (Hernández-Navarro 2023), Venezuela (Dennis 1970). Asia: Philippines (Long & Ahmad 1947, Wright 1987), India (Long & Ahmad 1947), Singapore (Calonge *et al.* 2013). Despite being one of the most recognizable species, it seems to be cryptically diverse. Morphologically, Wright (1987) described the verrucae of the exoperidium cells as being "like a puzzle", based on the holotype of *T. exasperatum* from Cuba and other specimens from American, African, and Asian countries, without providing measurements of the structures. Calonge *et al.* (2013) described "hyphae 3–6 µm, intermixed with sphaerocysts up to 15 µm in diameter, with intracellular brown pigment" from specimens from Singapore, which matches our materials and Wright's (1987); however, these materials have not been sequenced yet. Cabral *et al.* (2023) revised specimens from Brazil. They described a very similar new species, *T. paratyense*, which differs in having basidospores with a halo under light microscopy and helicoid morphology with uneven alveoli under SEM. They also sequenced specimens from Singapore identified as *T. ridleyi* and recognized as an independent species. Paloi *et al.* (2023) sequenced specimens identified as *T. exasperatum* from Thailand. However, these specimens were described with a circular ostiole and smaller basidiospores (3.70–7.25 × 3.50–6.66 µm). Additionally, the exoperidium verrucae was described as "composed of two types of hyphae: generative hyphae 2.3–3.0 µm wide, hyaline, branched, thin-walled, and skeletal hyphae 2.5–5.0 µm wide, yellowish, septate, branched, with a clamp-connection-like structure present". The combination of characters in the Thai materials does not match the holotype description, which is why they are designated as an affinis species to *T. exasperatum*. The identification of at least three species within the morphological concept of *T. exasperatum s.l.* Opens the hidden diversity of one of the most distinctive species in the genus, whose deeper morphological characterization expands the characters with taxonomic importance.

On *T. pulchellum*, this is a subcosmopolitan species commonly found in xeric habitats, recognizable by its combination of characters. The isolated verrucae of the basidiospores were considered a taxonomic character that distinguished it from *T. subfuscum* White (1901: 28), with basidiospores with fused verrucae, which was later synonymized with *T. pulchellum* based on DNA sequences (Jeppson *et al.* 2017, White 1901, Wright 1987). This species can be confused with *T. cyclophorum* and *T. striatum*, both of which have fibrillose to mammeliform ostioles with membranous exoperidium. However, they differ in having subreticulate and striate basidiospores, respectively, and the presence of microsclereids in *T. cyclophorum* (Cunningham 1925, Lloyd, 1906). In Mexico, *T. pulchellum* is reported from the states of Baja California, Chihuahua, CDMX, Coahuila, San Luis Potosí, Sonora, and Tamaulipas (Ayala *et al.* 1985, Calonge *et al.* 2004, Esqueda *et al.* 2004, 2006, 2012, Guzmán *et al.* 1992, Guzmán & Herrera 1969, 1973, Herrera 1959, Moreno *et al.* 2010).

Regarding *T. punctatum*, a new record for Mexico, the remaining Mexican materials accompanying the description of *T. obscurum* must be analyzed, as well as the holotype, since some inconsistencies exist in the descriptions. In the original description of *T. obscurum*, the basidiospores were described as: "yellowish-brown, subglobose to ellipsoidal, many deformed, apiculate, (4.6–) 5.5–6.1 (–7.6) × (4–) 5–5.5 (–6.7) µm, warty to spiny, with the warts and/or spines generally hyaline, forming short irregularly arranged ridges in some, and in others oriented like meridians without forming stretch marks". However, in the world monograph, Wright (1987) described the basidiospores of *T. obscurum* as follows: "verruculose, althought the verrucae are difficult to discern, brownish-yellow, episporium thick, 6.8–8.5 (10) µm diam; some very large ones mixed with smaller ones, may have large verrucae mixed with smaller ones, may have large verrucae mixed with smaller ones, granular under L.M; under SEM the ornamentation appears as relatively small, isolated, almost globose verrucae mixed with others anastomosed in short crest". Hence, the two different descriptions of the size and ornamentation of the basidiospores, in addition to the absence of such SEM images, make *T. obscurum* a doubtful species. On the other hand, the unpublished data of Honan (2021) describes specimens collected in 2015 from Colorado, USA, as "large, 6.7–8.7(9.8) µm, densely ornamented with very short, blunt or rounded, connected verrucae, that are nearly impossible to discern without SEM imaging. The ornamentation can be so dense and connected that individual verrucae are indiscernible. In areas of the spores with less dense verrucae, the ornamentation appears almost from reticulation". Nucleotide sequences reportedly support the independence of this species. However, the accession numbers are not provided, and the sequences remain unpublished. In addition, Jeppson *et al.* (2017) described the basidiospores of *T. punctatum* as presenting independent verrucae, while *T. fimbriatum* presents fused verrucae, although our material presents anastomosed verrucae forming irregular patterns, widening the morphological plasticity of *T. punctatum.* Nevertheless, *T. fimbriatum* and its former varieties are widely cited in Mexico, resulting in the need to review such collections.

Despite *T. rufum* being reported in Jalisco (Rodríguez Alcántar *et al.* 2024), our analysis invalidates this record, as the sequence does not match the holotype, but is more related to *T. verrucosum* and *T. squamosum.* In addition, their materials present a membranous exoperidium, a whitish gleba, basidiospores with fused spines, and two ranges of size were given (6–7.6 × 5.6–6.8 µm and 5.2–5.6 × 4.8–5.2 µm); no microsclereids were reported. Also, the details in the exoperidium show verrucae, a characteristic present in both *T. verrucosum* and *T. squamosum*, and not in *T. rufum*. On the other hand, they claimed to have obtained an alignment of 2269 bp, but all their deposited sequences are less than 200 bp from the 28S. In spite of their *T. striatum* and *T. frimbriatum* sequences grouping with the assigned species, that was not the case in *T. xerophillum,* and their materias present a coarser basidiosporal ornamentation than the type analyzed by Jeppson *et al.* (2017).

On *T. simulans*, Wright (1987) considered it a widely distributed species, but it was later considered a synonym of *T. beccarianum* (Altés & Moreno, 1993). However, molecular data supported their independence (Jeppson *et al.* 2017). Both names have been cited in Mexico: *T. simulans* in Aguascalientes, CDMX, Hidalgo, Morelos, and San Luis Potosí, while *T. beccarianum* is known from Sonora (Esqueda *et al.* 2000, 2004, Guzmán & Herrera 1973, Pardavé 1991, Wright *et al.* 1972). Such specimens must be sequenced in order to validate their identity. Regarding *T. elvirae*, initially described along with *T. brasiliense,* its ITS similarity is 98% with *T. simulan*s, and the ITS+LSU analysis does not entirely separate the species. At the same time, the three-gene phylogeny provides full support for its independence. In addition to molecular data, the doubtful original determination, and the fact that *T. brasiliense* has inconsistencies in the description and SEM characteristics of the holotype, we firmly believe the Mexican specimens represent a new species. *T. brasiliense* is also reported in Jalisco, Mexico (Sánchez-Jácome & Guzmán-Dávalos 2011); however, the record is in fact from an unpublished bachelor's thesis (Oliva 2000), which must be sequenced to confirm its conspecificity, which is why the species is not totally discarded from Mexico for the time being. Our results question the reliability of the use of the ITS barcode without additional markers. It has been pointed out that the ITS region is the barcode for fungi, but its resolution power is lower than protein-coding genes such as *RPB1* (Schoch *et al.* 2012) and *tef1-α* (Stielow *et al.* 2015). Although *tef1-α* was proposed as a secondary barcode, it presents difficulties in PCR amplification, whereas ITS can be amplified from environmental or degraded samples. Furthermore, the available primers for *tef1-α* are not universal for fungi, which complicates the barcoding labor. Some authors use a 3% threshold of sequence identity to determine conspecificity; despite this being mostly accurate for Basidiomycetes, the range is too high for some groups and too low for others (Begerow *et al.* 2010, Nilsson *et al.* 2008). Hence, the ITS by itself may not be enough to recognize closely related species in *Tulostoma*; therefore, the *tef1-α* must be included with most of the possible samples to assess true diversity and phylogenetic relationships.

On the main Clades, most of them match those of Jeppson *et al.* (2017), with some exceptions. Clades 1 and 2 are considered here as a single Clade (Obesum Clade). In the original phylogeny, Clade 2 was related to Clade 1 but was unsupported and composed of a single species (*T. punctatum*). Clade 3 is equivalent to the Fimbriatum Clade. Clade 4, composed of *T.* sp 4 and 5, was not recovered in this study. It was mentioned that Clade 4 had no support. The ITS+28S considered each species as a single branch, while the concatenated tree considered *T.* sp. 4 closer to the Fimbriatum clade with medium support (BS/PP=49/0.7), and *T.* sp. 5 to the Squamosum clade with high support (BS/BP=99/0.9). Despite not being fully described, *T.* sp. 4 was mentioned to be similar to *T. fimbriatum* and *T.* sp. 5, characterized by a circular mouth and echinate basidiospores (Jeppson, M. *et al.* 2017), which matches some members of the Squamosum Clade. Hence, the concatenated analysis presented here is congruent with the morphological characteristics mentioned.

Clade 5 is equivalent to the Kotlabae Clade. However, it is currently composed mainly of undescribed species. As mentioned previously, the materials of *T. aff. macrosporum* from Mexico does not match those from Australia (Varga *et al.* 2019). However, the Australian materials were used in a mega-phylogeny, but not described, and their identity cannot be confirmed. In addition to not matching molecularly, the holotype is defined as presenting an ochraceous gleba with a purplish hue, a characteristic not reported in any of the Mexican materials reported as *T. macrosporum* (Esqueda *et al.* 2004), which may suggest cryptic diversity within this name. In Mexico, this species has been reported in the arid and semiarid zones of the states of Baja California, Chihuahua, Nuevo León, Sonora, and Zacatecas (Calonge *et al.* 2004, Esqueda *et al.* 2004, Guzmán *et al.* 1992, Moreno *et al.* 2010).

The Laceratum Clade and the Pseudopulchellum Clade were previously considered as a single clade (Clade 6) by Jeppson *et al.* (2017); however, with the addition of Hungarian (Finy *et al.*, 2023) and Mexican materials, the morphological characteristics between the members of each clade have become clearer. While members of the Laceratum Clade all present ostioles that tend to be indefinite, pigmented and dissarticulated capillitium, and concolorous smooth spores, the members of the Pseudopulchellum Clade present definite ostioles, either fibrillose or tubular, and ornamented basidiospores that vary from asperulate (i.e., *T. pannonicum*, *T. pseudopulchellum*) to subreticulate (i.e., *T. teophilii*, *T. catimbauense*) with hyaline to subhialyne capillitium, with articulated and swollen septa.

The here-named Brumale Clade corresponds with Clade 10 of Jeppson *et al.* (2017), which was described as unsupported (69/-), whereas it is recovered here with medium-high support (82/0.52). The Squamosum Clade corresponds with Clade 11; however, two main subclades are recovered, one with most of the species recognized in this clade, and another with *T. deltaconcaum* and *T. subreticulatum*. Although it was mentioned that *T. subreticulatum* varied more than 10% from the closest member of Clade 11, and proposed provisionally as Clade 12 (Hernández-Navarro & Cappello-García 2024), the concatenated analysis presented here recovered *T. subreticulatum* as a basal taxon of Squamosum Clade. However, *T. deltaconcavum* lacks LSU and *tef1-a* sequences.

Finally, the *T. exasperatum* specimens and their allies are grouped in a distinct new clade, composed of species with truly reticulate basidiospores and a verrucose exoperidium. This clade was recovered as an independent branch, related to *T. brumale* and allies without support by Paloi *et al.* (2023), while in Cabral *et al.* (2023), the clade was recovered as a polytomy. Hence, the phylogenetic relationship of this clade within the genus is still unknown.

Integrating morphological and molecular data has partially clarified the taxonomy and phylogeny of *Tulostoma*. Jeppson *et al.* (2017) reported the exclusion of 335 characters considered ambiguous data from the ITS1 and ITS2 regions. This strategy is often employed to enhance the support values in phylogenetic trees; however, it may result in the loss of important information. Nevertheless, even when included here, most of the deep branches still require full support. Low support values are related to evolutionary processes such as low phylogenetic signal, incomplete lineage sorting, rapid speciation, recent radiation, long-branch attraction, and introgression of alleles across species boundaries (e.g., incomplete reproductive isolation) due to different selective processes (Bergsten 2005, Degnan & Rosenberg 2009, Mallet *et al.* 2016, Morando *et al.* 2004, Whitfield & Lockhart 2007). The incorporation of material from other continents should clarify the infrageneric relationships of the clades, as most sequenced specimens are Palearctic, mostly from semiarid areas of Europe, with a few Neoarctic, Neotropical, and Indomalayan sequences, but none from the Afrotropical, Australasian, and Oceanian biogeographic realms.

Some collections here examined were obtained several years ago and have not been re-recorded anywhere in Mexico. On the one hand, research on the genus is generally scarce; on the other hand, climate change, deforestation, urbanization, and other anthropogenic factors could directly impact the diversity of the genus. Wright (1987) considered this genus to be sensitive to disturbance, mentioning that once the vegetation is altered, no fructifications are seen for up to seven years. In contrast, in Sonora, Mexico, 12% of the Tulostomataceae specimens were collected in perturbed environments, such as urban zones, agricultural lands, and cultivated grasslands (Hernández-Navarro *et al.* 2020). This percentage includes: *Battarrea phalloide*s (1801: 1), *Battarreoides diguetii* (1961: 32) (Heim, R. & Herrera, T. 1961), *T. albicans*, *T. chudaei* Pat. (1907: 84), *T. cretaceum*, *T. fimbriatum*, *T. laceratum*, *T. leiosporum* R.E. Fr. (1908: 28), *T. macrosporum*, *T. mohavei* Lloyd (1920: 992), *T. nanum* (Pat.) J.E. Wright (1987: 113), *T. obesum*, *T. pulchellum,* and *T. xerophilum* (Fries 1908, Lloyd 1920, Patouillard 1907, Persoon 1801). These species are Sonora's most abundant taxa, although slight morphological variations were observed in most of the mentioned taxa. Despite this, the identity of these collections based on DNA sequences is yet to be determined, since it was based only on morphology.

Notably, despite being easily overlooked species, since they grow in habitats not commonly visited by mycologists, some species of *Tulostoma* are considered rare and threatened. In Slovakia, *T. kotlabae* is considered endangered. In the Czech Republic, *T. pulchellum* and *T. simulans* (=*T. moravecii* (1958: B) in the text) are considered extinct, while *T. melanocyclum* is endangered (Holec & Beran 2006, Petri 1904, Pouzar 1958). In Austria, six species of *Tulostoma* are on the national red list; these include *T. fulvellum* Bres. (1904: 425) (=*T. armillatum* Bres (1904: 2) in the text), *T. kotlabae*, *T. melanocyclum,* and *T. simulans* (=*T. moravecii* in the text) are considered strongly endangered, and *T.*

fimbriatum and *T. squamosum* are considered endangered (Krisai-Greilhuber, I. 1999). *T. dominguezia*e was described from *Polyepis australis* Ruiz & Pav. Woodlands, a threatened ecosystem, is the only species of *Tulostoma* considered critically endangered in Argentina (Hernández Caffot *et al.* 2011, Hernández-Caffot *et al.* 2023, Kuhar *et al.* 2020). In Mexico, there are rare taxa, such as *T. portoricense* J.E. Wright (1987: 218) and *T. gracilipe*s J.E. Wright (1987: 125), which have only been recorded worldwide on two or three occasions, respectively (Esqueda-Valle *et al.* 1998, Piña *et al.* 2010); however, the conservation status of most of the species of this genus in Mexico is unknown. Finally, with the new records presented here and the addition of new species, a total of 56 *Tulostoma species* are currently known in Mexico. It is essential to sequence more specimens from diverse biomes and previously unexplored areas to accurately assess the true diversity of the genus and deepen our understanding of its phylogeny.

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Bibliography

Akata, İ., Sahi̇n, E., Altuntaş, D. & Kabaktepe, Ş. (2021) Two New Additions to Turkish *Tulostoma*. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi* 24: 1104–1110. https://doi.org/10.18016/ksutarimdoga.vi.839465

Altés, A. & Moreno, G. (1993) *Tulostoma beccarianum* Bres., the correct name for *T. simulans* Lloyd. *Mycotaxon* 48: 223–227.

Ayala, N., Ochoa, C. & Guzmán, G. (1985) Los hongos de la Península de Baja California, II. Las especies conocidas de *Tulostoma*. *Revista Mexicana de Micología* 1: 465–469.

Baseia, I.G. & Galvão, T.C. de O. (2002) Some interesting Gasteromycetes (Basidiomycota) in dry areas from northeastern Brazil. *Acta botanica Brasilica* 16: 1–8. https://doi.org/10.1590/s0102-33062002000100002

Baseia, I.G. & Milanez, A.I. (2002) *Tulostoma* Persoon (Gasteromycetes) from the cerrado region, State of São Paulo, Brazil. *Acta botanica Brasilica* 16: 09–14. https://doi.org/10.1590/s0102-33062002000100003

Begerow, D., Nilsson, H., Unterseher, M. & Maier, W. (2010) Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied microbiology and biotechnology* 87: 99–108. https://doi.org/10.1007/s00253-010-2585-4

Bergsten, J. (2005) A review of long-branch attraction. *Cladistics: the international journal of the Willi Hennig Society* 21: 163–193. https://doi.org/10.1111/j.1096-0031.2005.00059.x

Bononi, V.L., Guzmán, G. & Capelari, M. (1984) Basidiomycetos do Parque Estadual da Ilha do Cardoso. V: Gasteromycetos. *Rickia* 11: 91–97.

Cabral, T.S., Silva, B.D.B. da, Vargas-Isla, R., Oliveira, J.J.S. de, Ferreira, J.A. da S., Castro, L., Martín, M.P. & Ishikawa, N.K. (2023) Diversity of Neotropical stalked-puffball: Two new species of *Tulostoma* with reticulated spores. *PloS one* 18: e0294672. https://doi.org/10.1371/journal.pone.0294672

Calonge, F.D. (2000) Validation or confirmation of some new taxa recently published. *Mycological Research* 103: 301–302.

Calonge, F.D. & Almeida, M.G. (1992) *Tulostoma lusitanicum sp. nov.* (Gasteromycetes). *Bulletin de la Sociedad Micológica de Madrid* 16: 109–113.

Calonge, F.D., Guzmán, G. & F. Ramírez-Guillén, G. (2004) Observaciones sobre los Gasteromycetes de México depositados en los herbarios XAL y XALU. *Boletin de la Sociedad Micológica de Madrid* 28: 337–370.

Calonge, F.D., Llimona, X. & Martin, M.P. (2007) Nuevos datos sobre el género *Tulostoma* (Gasteromycetes) en España. IV. *Revista Catalana de Micología* 29: 1–16.

Calonge, F.D., Vidal, J.M. & Altés, A. (2013) *Tulostoma exasperatum* Mont., from Singapore. *Boletin de la Sociedad Micológica de Madrid* 37: 59–63.

Cooke, M.C. & Ellis, J.B. (1878) New Jersey fungi. *Grevillea* 6: 81–96.

Cunningham, G.H. (1925) Gasteromycetes of Australasia II. A revision of the genus *Tulostoma*. *Proceedings of the Linnean Society of New South Wales* 50: 245–259.

Degnan, J.H. & Rosenberg, N.A. (2009) Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in ecology & evolution* 24: 332–340.
https://doi.org/10.1016/j.tree.2009.01.009

Dennis, R.W.G. (1970) *Fungus flora of Venezuela and adjacent countries*. H.M. Stationery Office, London, 531 pp.

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin* 19: 11–15.

Esqueda, M., Coronado, M., Sánchez, A., Pérez-Silva, E. & Herrera, T. (2006) Macromycetes of pinacate and great altar desert biosphere reserve, Sonora, Mexico. *Mycotaxon* 95: 81–90.

Esqueda, M., Gutiérrez, A., Coronado, M.L., Lizárraga, M., Raymundo, T. & Valenzuela, R. (2012) Distribución de algunos hongos gasteroides (Agaricomycetes) en la planicie central del Desierto Sonorense. *Revista Mexicana de Micología* 36: 1–8.

Esqueda, M., Moreno, G., Perez-Silva, E., Sanchez, A. & Altes, A. (2004) The genus *Tulostoma* in Sonora, Mexico. *Mycotaxon* 90: 409–422.

Esqueda, M., Pérez-Silva, E., Herrera, T., Coronado, M. & Estrada-Torres, A. (2000) Composición de gasteromicetos en un gradiente de vegetación de Sonora, México. *Anales del Instituto de Biología. Serie Botánica* 71: 39–62.

Esqueda-Valle, M., Pérez-Silva, E., Herrera, T., Altés, A. & Moreno, G. (1998) *Tulostoma portoricense* (Tulostomatales, Gasteromycetes) from Mexico. *Mycotaxon* 68: 499–503.

Finy, P., Jeppson, M., Knapp, D.G., Papp, V., Albert, L., Ölvedi, I., Bóka, K., Varga, D., Kovács, G.M. & Dima, B. (2023) Exploring diversity within the genus *Tulostoma* (Basidiomycota, Agaricales) in the Pannonian sandy steppe: four fascinating novel species from Hungary. *MycoKeys* 100: 153–170.
https://doi.org/10.3897/mycokeys.100.112458

Fries, E.M. (1829) *Systema Mycologicum*. Sumptibus Ernesti Mauritii, Greifswald, 259 pp.

Fries, R.E. (1908) Über einige Gasterinyceten aus Bolicia und Argentinien. *Arkiv för botanik* 8: 1–31.

Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular ecology* 2: 113–118.
https://doi.org/10.1111/j.1365-294x.1993.tb00005.x

Gorbunova, I.A. & Rebriev, Y.A. (2016) New data to biota Gasteromycetes of the Altai-Sayan mountain-region. *Rastitelnyy mir Aziatskoy Rossii [Plant Life of Asian Russia]* 2: 3–7.

Guzmán, G. & Herrera, T. (1969) Macromicetos de las zonas áridas de México, II Gasteromicetos. *Anales del Instituto de Biología, UNAM, Serie Botánica* 40: 1–92.

Guzmán, G. & Herrera, T. (1973) Especies de macromicetos de México citadas IV. Gasteromicetos. *Boletín de la Sociedad Mexicana de Micología* 7: 105–119.

Guzmán, G., Montoya, L. & Bandala, V. (1992) Adiciones al conocimiento del género *Tulostoma* (basidiomycotina, Gasteromycetes) en México y descripción de una nueva especie. *Boletín de la Sociedad Argentina de Botánica* 28: 113–121.

Heim, R. & Herrera, T. (1961) Una nueva especie de *Podocrea* y una nueva combinación para el nombre de un hongo mexicano del género *Battarreoides*. *Anales del Instituto de Biología de la Universidad Nacional Autónoma de México* 32: 29–31.

Hernández Caffot, M.L., Domínguez, L.S. & Hosaka, K. (2011) *Tulostoma domingueziae sp. nov.* from *Polylepis australis* woodlands in Córdoba Mountains, central Argentina. *Mycologia* 105: 1047–1054.
https://doi.org/10.3852/10-266

Hernández-Caffot, M.L., Vaira, M. & Domínguez, L.S. (2023) Hongos de Argentina evaluados por la UICN (Unión Internacional para la Conservación de las Especies y status de *Rickiella edulis* basada en un nuevo registro en Jujuy, Argentina. *Lilloa* 60: 287–308.
https://doi.org/10.30550/j.lil/1853

Hernández-Navarro, E. (2023) First record of *Tulostoma exasperatum* (Agaricomycetes, Agaricales) in Mexico. *Acta Botanica Mexicana* 130: e2171.
https://doi.org/10.21829/abm130.2023.2171

Hernández-Navarro, E. & Cappello-García, S. (2024) *Tulostoma subreticulatum* (Agaricomycetes, Agaricales): a new species of stalked

puffball from Mexico. *Phytotaxa* 653: 101–114.
https://doi.org/10.11646/phytotaxa.653.2.1

Hernández-Navarro, E., Gutiérrez, A., Coronado, M.L., Álvarez-Bajo, O., Andrade, S., Barredo-Pool, F. & Esqueda, M. (2020) Diversity, morphological variability, and distribution of tulostomataceous fungi (Agaricomycetes) in Sonora, Mexico. *Revista Mexicana de Biodiversidad* 91: e913246.
https://doi.org/10.22201/ib.20078706e.2020.91.3246

Hernández-Navarro, E., Gutiérrez, A., Ramírez-Prado, J.H., Sánchez-Teyer, F. & Esqueda, M. (2018) *Tulostoma rufescens sp. nov.* from Sonora, Mexico. *Mycotaxon* 133: 459–471.
https://doi.org/10.5248/133.459

Hernández-Navarro, E., Gutiérrez-Saldaña, A., Barredo-Pool, F. & Esqueda, M. (2015) Especies de *Tulostoma* (Basidiomycetes, Agaricomycetes) en un matorral espinoso de Sonora, México. *Revista Mexicana de Micología* 41: 65–72.

Herrera, T. (1959) *Battarraea* y *Tylostoma* en el Valle de México. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México. Serie botánica* 1: 21–33.

Holec, J. & Beran, M. (2006) Červený seznam hub (makromycetů) České republiky [Red list of fungi (macromycetes) of the Czech Republic]. *Příroda* 24: 282.

Honan, A.H. (2021) An Evolutionary and Ecological Investigation into the Enigmatic Stalked Puffball Genus *Tulostoma*. Doctorate Dissertation. University of Washington, 218 pp.

Hussain, S., Yousaf, N., Afshan, N.-U.-S., Niazi, A.R., Ahmad, H. & Khalid, A.N. (2016) *Tulostoma ahmadii sp. nov.* and *T. squamosum* from Pakistan. *Turkish journal of botany* 40: 218–225.
https://doi.org/10.3906/bot-1501-9

Index Fungorum (2025) Index Fungorum. Available from: http://www.indexfungorum.org/Names (accessed August 2025)

Jeppson, M., Altés, A., Moreno, G., Nilsson, R.H., Loarce, Y., Bustos, A. & Larsson, E. (2017) Unexpected high species diversity among European stalked puffballs–a contribution to the phylogeny and taxonomy of the genus *Tulostoma* (Agaricales). *Mykokeys* 21: 33–88.
https://doi.org/10.3897/mycokeys.21.12176

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A. & Jermiin, L.S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature methods* 14: 587–589.
https://doi.org/10.1038/nmeth.4285

Katoh, K., Misawa, K., Kuma, K.-I. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research* 30: 3059–3066.
https://doi.org/10.1093/nar/gkf436

Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in bioinformatics* 20: 1160–1166.
https://doi.org/10.1093/bib/bbx108

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 30: 772–780.
https://doi.org/10.1093/molbev/mst010

Kers, L.E. (1978) *Tulostoma niveum* sp. nov. (Gasteromycetes) described from Sweden. *Botaniska Notiser* 131: 411–417.

Kim, C.S., Jo, J.W., Kwag, Y.N., Sung, G.H., Han, J.G., Kim, S.Y. & Oh, S. (2016) Two new *Lycoperdon* species collected from Korea: *L. albiperidium* and *L. subperlatum spp. nov*. *Phytotaxa* 260: 101–115.
https://doi.org/10.11646/phytotaxa.260.2.1

Kornerup, A. & Wanscher, J.H. (1978) *Methuen handbook of colour*. Eyre Methuen, London, UK, 252 pp.

Krisai-Greilhuber, I. (1999) Rote Liste gefährdeter Großpilze Österreichs 2. Fassung. *In*: Niklfeld, H. (Ed.) *Rote Listen gefährdeter Pflanzen Österreichs, 2. Auflage.* Grüne Reihe des Bundesministeriums für Umwelt, Jugend und Familie, pp. 229–266.

Kropp, B.R., Albee-Scott, S., Castellano, M.A. & Trappe, J.M. (2012) *Cryptolepiota*, a new sequestrate genus in the Agaricaceae with evidence for adaptive radiation in western North America. *Mycologia* 104: 164–174.
https://doi.org/10.3852/11-046

Kuhar, F., Greslebin, A. & Pfister, D. (2020) *Tulostoma domingueziae. The IUCN Red List of Threatened Species 2020: e.T172742153A172861262*.
https://doi.org/10.2305/IUCN.UK.2020-2.RLTS.T172742153A172861262.en

Larsson, A. (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30: 3276–3278.
https://doi.org/10.1093/bioinformatics/btu531

Lebel, T. & Vellinga, E.C. (2013) Description and affinities of a sequestrate *Lepiota* (Agaricaceae) from Australia. *Mycological progress* 12: 525–532.

https://doi.org/10.1007/s11557-012-0859-1

Li, J.X., Cao, B., Phurbu, D., He, M., Zhu, X.Y., Parra, L.A. & Zhao, R.L. (2024) The revision of the taxonomic system of Lycoperdaceae. *Mycosphere, journal of fungal biology* 15: 4919–5016.
https://doi.org/10.5943/mycosphere/15/1/22

Lima, A., Melanda, G., Marinho, P., Martín, M.P. & Baseia, I. (2023) Two new species of *Tulostoma* (Agaricales, Basidiomycota) from the Neotropics. *Turkish journal of botany* 47: 399–407.
https://doi.org/10.55730/1300-008x.2777

Lloyd, C.G. (1906) The Tylostomeae. *Mycological Writings* 2: 1–28.

Lloyd, C.G. (1920) Mycological notes 64. *Mycological Writings* 64: 985–1029.

Long, W.H. (1944) Studies in the gasteromycetes X. Seven new species of *Tylostoma*. *Mycologia* 36: 318–339.
https://doi.org/10.1080/00275514.1944.12017555

Long, W.H. (1946) Studies in the gasteromycetes XII. Five species of *Tylostoma* with membranous exoperidia. *Mycologia* 38: 77–90.
https://doi.org/10.1080/00275514.1946.12024041

Long, W.H. (1947) Studies in the Gasteromycetes: XV. Notes on new or rare species of *Tylostoma*. *Lloydia* 10: 115–135.

Long, W.H. & Ahmad, S. (1947) The genus *Tylostoma* in India. *Farlowia* 3: 225–267.
https://doi.org/10.5962/p.316027

Mallet, J., Besansky, N. & Hahn, M.W. (2016) How reticulated are species? *BioEssays: news and reviews in molecular, cellular and developmental biology* 38: 140–149.
https://doi.org/10.1002/bies.201500149

Masse, G.A. monograph of British Gasteromycetes. *Annals of botany* 4: 1–103.
https://doi.org/10.1093/oxfordjournals.aob.a090544

Media Cybernetics (2024) *Image-Pro Plus v. 7.0*. Rockville, EUA. [https://mediacy.com/image-pro/]

Minh, B.Q., Nguyen, M.A.T. & von Haeseler, A. (2013) Ultrafast approximation for phylogenetic bootstrap. *Molecular biology and evolution* 30: 1188–1195.
https://doi.org/10.1093/molbev/mst024

Montagne, C. (1837) Centurie de plantes cellulaires exotiques nouvelles. *Annales des sciences naturelles* 8: 345–370.

Morando, M., Avila, L.J., Baker, J. & Sites Jr, J.W. (2004) Phylogeny and phylogeography of the *Liolaemus darwinii* complex (Squamata: Liolaemidae): evidence for introgression and incomplete lineage sorting. *Evolution; international journal of organic evolution* 58: 842–861.
https://doi.org/10.1111/j.0014-3820.2004.tb00416.x

Moreno, G., Altés, A., Ochoa, C. & Wright, J.E. (1995) Contribution to the study of the Tulostomataceae in Baja California, Mexico. I. *Mycologia* 87: 96–120.
https://doi.org/10.1080/00275514.1995.12026509

Moreno, G., Altés, A. & Wright, J. (1992) *Tulostoma pseudopulchellum sp. nov.* (Tulostomatales, Gasteromycetes) and allied species. *Mycotaxon* 43: 479–486.

Moreno, G., Heykoop, M., Esqueda, M. & Olariaga, I. (2015) Another lineage of secotioid fungi is discovered: *Psathyrella secotioides sp. nov.* from Mexico. *Mycological progress* 14.
https://doi.org/10.1007/s11557-015-1057-8

Moreno, G., Lizárraga, M., Esqueda, M. & Coronado, M.L. (2010) Contribution to the study of gasteroid and secotioid fungi of Chihuahua, Mexico. *Mycotaxon* 112: 291–315.
https://doi.org/10.5248/112.291

Morgan, A.P. (1890) North American fungi. The gasteromycetes III. *The Journal of the Cincinnati Society of Natural History* 12: 8–22.

Niazi, A.R., Ghafoor, A., Afshan, N. & Moreno, G. (2022) *Tulostoma loonbanglaense*: A new species from Azad Jammu and Kashmir using light and scanning electron microscopy and DNA barcoding technique. *Microscopy research and technique* 85: 3720–3725.
https://doi.org/10.1002/jemt.24240

Nilsson, R.H., Kristiansson, E., Ryberg, M., Hallenberg, N. & Larsson, K.H. (2008) Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary bioinformatics online* 4: 193–201.
https://doi.org/10.4137/EBO.S653

Oliva, M.A. (2000) *Contribución al conocimiento de los hongos (Macromicetos) de La Laguna de Sayula, Jalisco.* Bachelor thesis. Universidad de Guadalajara, Zapopan, México.

Paloi, S., Suwannarach, N., Kumla, J., Phonrob, W., Karunarathna, S.C. & Lumyong, S. (2023) An update on species diversity, distribution and sequence data of *Tulostoma* in Asia with the addition of *Tulostoma exasperatum*, a new record for Thailand. *Chiang Mai Journal of Science* 50: 1–14.

https://doi.org/10.12982/CMJS.2023.018

Pardavé, L.M. (1991) Gasteromicetos del Estado de Aguascalientes. *Scientia fungorum* 7: 71–77.

Patouillard, N. (1907) Champignons nouveaux du Tonkin. *Bulletin trimestriel de la Société mycologique de France* 23: 69–79.

Peck, C.H. (1906) New species of fungi. *Bulletin of the Torrey Botanical Club* 23: 411–420. https://doi.org/10.2307/2477789

Persoon, C.H. (1794) Dispositio methodica fungorum in classes, ordines, Familias et Genera. *Neues Magazin für die Botanik, Ziegler u. Söhne, Zürich*: 63–128.

Persoon, C.H. (1801) *Synopsis Methodica Fungorum (A Methodical Synopsis of the Fungi)*. Henricus Dieteric, Germany, Göttingen.

Petri, L. (1904) Sul valore diagnostico del capillizio nel genere "*Tylostoma*" Pers. *Annales Mycologici* 2: 412–438.

Piña, C., Esqueda, M., Altés, A. & Gutierrez, A. (2010) First record of *Tulostoma gracilipes* (Agaricales, Agaricaceae) for the Americas. *Mycotaxon* 113: 371–376. https://doi.org/10.5248/113.371

Pouzar, Z. (1958) *Tulostoma*. *In*: Pilat, A. (ed.) *Flora CSR: Gasteromycetes*. Prace Ceskoslovenské Akademie Ved. Praha, pp. B: 589–613; 810–815.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarization in Bayesian phylogenetics using tracer 1.7. *Systematic biology* 67: 901–904. https://doi.org/10.1093/sysbio/syy032

Rehner, S.A. & Buckley, E. (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97: 84–98. https://doi.org/10.1080/15572536.2006.11832842

Rick, J. (1961) Basidiomycetes eubasidii in Rio Grande do Sul-Brasilia 6. *Iheringia Sér Bot* 9: 451–479.

Rodríguez Alcántar, O., Figueroa-García, D., Ruíz-Sanchez, E., Zamora-Tavares, P., Herrera-Fonseca, M.J., Esqueda, M. & Vargas-Ponce, O. (2024) Advances in the phylogeny of the genus *Tulostoma* in Mexico, new records for the country, Jalisco and Zacatecas. *e-CUCBA* 12: 34–51. https://doi.org/10.32870/e-cucba.vi24.375

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology* 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Rusevska, K., Calonge, F.de D., Karadelev, M. & Martín, M.P. (2019) Fungal DNA barcode (ITS nrDNA) reveals more diversity than expected in *Tulostoma* from Macedonia. *Turkish journal of botany* 43: 102–115. https://doi.org/10.3906/bot-1804-38

Saccardo, P.A. (1887) *Sylloge Fungorum 5: i–iii,* Saccardo, P.A., Italy, Padua, 1146 pp.

Saccardo, P.A. (1888) *Sylloge Fungorum 7 (1–2): i*. Saccardo, P.A., Italy, Pavia, 882 pp.

Sánchez-Jácome, M.R. & Guzmán-Dávalos, L. (2011) Hongos citados para Jalisco, II. *Ibugana: Boletín del Instituto de Botánica* 1: 25–60.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Bolchacova, E., Voigt, K., Crous, P.W., Miller, A.N., Wingfield, M.J., Aime, M.C., An, K.-D., Bai, F.-Y., Barreto, R.W., Begerow, D., Bergeron, M.-J., Blackwell, M., Boekhout, T., Bogale, M., Boonyuen, N., Burgaz, A.R., Buyck, B., Cai, L., Cai, Q., Cardinali, G., Chaverri, P., Coppins, B.J., Crespo, A., Cubas, P., Cummings, C., Damm, U., de Beer, Z.W., de Hoog, G.S., Del-Prado, R., Dentinger, B., Diéguez-Uribeondo, J., Divakar, P.K., Douglas, B., Dueñas, M., Duong, T.A., Eberhardt, U., Edwards, J.E., Elshahed, M.S., Fliegerova, K., Furtado, M., García, M.A., Ge, Z.-W., Griffith, G.W., Griffiths, K., Groenewald, J.Z., Groenewald, M., Grube, M., Gryzenhout, M., Guo, L.-D., Hagen, F., Hambleton, S., Hamelin, R.C., Hansen, K., Harrold, P., Heller, G., Herrera, C., Hirayama, K., Hirooka, Y., Ho, H.-M., Hoffmann, K., Hofstetter, V., Högnabba, F., Hollingsworth, P.M., Hong, S.-B., Hosaka, K., Houbraken, J., Hughes, K., Huhtinen, S., Hyde, K.D., James, T., Johnson, E.M., Johnson, J.E., Johnston, P.R., Jones, E.B.G., Kelly, L.J., Kirk, P.M., Knapp, D.G., Kõljalg, U., Kovács, G.M., Kurtzman, C.P., Landvik, S., Leavitt, S.D., Liggenstoffer, A.S., Liimatainen, K., Lombard, L., Luangsa-ard, J.J., Lumbsch, H.T., Maganti, H., Maharachchikumbura, S.S.N., Martin, M.P., May, T.W., McTaggart, A.R., Methven, A.S., Meyer, W., Moncalvo, J.-M., Mongkolsamrit, S., Nagy, L.G., Nilsson, R.H., Niskanen, T., Nyilasi, I., Okada, G., Okane, I., Olariaga, I., Otte, J., Papp, T., Park, D., Petkovits, T., Pino-Bodas, R., Quaedvlieg, W., Raja, H.A., Redecker, D., Rintoul, T.L., Ruibal, C., Sarmiento-Ramírez, J.M., Schmitt, I., Schüßler, A., Shearer, C., Sotome, K., Stefani, F.O.P., Stenroos, S., Stielow, B., Stockinger, H., Suetrong, S., Suh, S.-O., Sung, G.-H., Suzuki, M., Tanaka, K., Tedersoo, L., Telleria, M.T., Tretter, E., Untereiner, W.A., Urbina, H., Vágvölgyi, C., Vialle, A., Vu, T.D., Walther, G., Wang, Q.-M., Wang, Y., Weir, B.S., Weiß, M., White, M.M., Xu, J., Yahr, R., Yang, Z.L., Yurkov, A., Zamora, J.-C., Zhang, N., Zhuang, W.-Y. & Schindel, D. (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* 109: 6241–6246.

https://doi.org/10.1073/pnas.1117018109

Schubert, H. & Specht, P. (2013) Seltene Gasteromyceten in Deutschland IV – *Tulostoma winterhoffii*, ein neuer Stielbovist. *Zeitschrift für Mykologie* 79: 417–430.

SEMARNAT (2003) Informe de la Situación del Medio Ambiente en México. *Procuraduría Ambiental y del Ordenamiento Territorial de la Ciudad de México*. Available from: https://paot.org.mx/centro/ine-semarnat/informe02/estadisticas_2000/informe_2000/03_Suelos/3.3_Fragiles/index.htm#desertificacion (accessed 16 July 2024)

SEMARNAT (2006) Programa Nacional Manejo Sustentable de Tierras. [https://www.gob.mx/cms/uploads/attachment/file/31167/pnacdd.pdf]

Spegazzini, C. (1927) Gasteromycetas Argentinas. *Physis* 8: 421–435.

Stielow, J.B., Lévesque, C.A., Seifert, K.A., Meyer, W., Iriny, L., Smits, D., Renfurm, R., Verkley, G.J.M., Groenewald, M., Chaduli, D., Lomascolo, A., Welti, S., Lesage-Meessen, L., Favel, A., Al-Hatmi, A.M.S., Damm, U., Yilmaz, N., Houbraken, J., Lombard, L., Quaedvlieg, W., Binder, M., Vaas, L.A.I., Vu, D., Yurkov, A., Begerow, D., Roehl, O., Guerreiro, M., Fonseca, A., Samerpitak, K., van Diepeningen, A.D., Dolatabadi, S., Moreno, L.F., Casaregola, S., Mallet, S., Jacques, N., Roscini, L., Egidi, E., Bizet, C., Garcia-Hermoso, D., Martín, M.P., Deng, S., Groenewald, J.Z., Boekhout, T., de Beer, Z.W., Barnes, I., Duong, T.A., Wingfield, M.J., de Hoog, G.S., Crous, P.W., Lewis, C.T., Hambleton, S., Moussa, T.A.A., Al-Zahrani, H.S., Almaghrabi, O.A., Louis-Seize, G., Assabgui, R., McCormick, W., Omer, G., Dukik, K., Cardinali, G., Eberhardt, U., de Vries, M. & Robert, V. (2015) One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia-Molecular Phylogeny and Evolution of Fungi* 35: 242–263.
https://doi.org/10.3767/003158515X689135

Varga, T., Krizsán, K., Földi, C., Dima, B., Sánchez-García, M., Sánchez-Ramírez, S., Szöllősi, G.J., Szarkándi, J.G., Papp, V., Albert, L., Andreopoulos, W., Angelini, C., Antonín, V., Barry, K.W., Bougher, N.L., Buchanan, P., Buyck, B., Bense, V., Catcheside, P., Chovatia, M., Cooper, J., Dämon, W., Desjardin, D., Finy, P., Geml, J., Haridas, S., Hughes, K., Justo, A., Karasiński, D., Kautmanova, I., Kiss, B., Kocsubé, S., Kotiranta, H., LaButti, K.M., Lechner, B.E., Liimatainen, K., Lipzen, A., Lukács, Z., Mihaltcheva, S., Morgado, L.N., Niskanen, T., Noordeloos, M.E., Ohm, R.A., Ortiz-Santana, B., Ovrebo, C., Rácz, N., Riley, R., Savchenko, A., Shiryaev, A., Soop, K., Spirin, V., Szebenyi, C., Tomšovský, M., Tulloss, R.E., Uehling, J., Grigoriev, I.V., Vágvölgyi, C., Papp, T., Martin, F.M. & Nagy, L.G. (2019) Megaphylogeny resolves global patterns of mushroom evolution. *Nature ecology & evolution* 3: 668–678.
https://doi.org/10.1038/s41559-019-0834-1

Vlasenko, V.A. & Vlasenko, A.V. (2023) A new species of *Tulostoma* genus from Siberia. *Phytotaxa* 600: 25–34.
https://doi.org/10.11646/phytotaxa.600.1.4

White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A guide to methods and applications* 18: 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1

White, V.S. (1901) The Tylostomaceae of North America. *Bulletin of the Torrey Botanical Club* 28: 421–444.
https://doi.org/10.2307/2478590

Whitfield, J.B. & Lockhart, P.J. (2007) Deciphering ancient rapid radiations. *Trends in ecology & evolution* 22: 258–265.
https://doi.org/10.1016/j.tree.2007.01.012

Wong, T.K.F., Ly-Trong, N., Ren, H., Baños, H., Roger, A.J., Susko, E., Bielow, C., De Maio, N., Goldman, N. & Hahn, M.W. (2025) IQ-TREE 3: Phylogenomic Inference Software using Complex Evolutionary Models. [https://iqtree.github.io/]
https://doi.org/10.32942/X2P62N

Wright, J.E. (1983) *Tulostoma exasperatosporum*, a new species with reticulate spores from South Africa. *South African Journal of Botany* 2: 126–128.
https://doi.org/10.1016/S0022-4618(16)30126-7

Wright, J.E. (1987) *The Genus* Tulostoma *(Gasteromycetes)—A World Monograph*. J. Cramer, 338 pp.

Wright, J.E., Herrera, T. & Guzmán, G. (1972) Estudios sobre el género *Tulostoma* en México (Fungi, Gasteromycetes). *Ciencia* 27: 109–122.