Tulostoma subreticulatum (Agaricomycetes, Agaricales): a new species of stalked puffball from Mexico

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Abstract

Tulostoma subreticulatum is a new species to science. This species is characterized by its small to medium-size basidiomes, tubular stoma, verrucose exoperidium with flat and angulose verrucae up to 0.8 mm, reddish-brown endoperidium, and subreticulated 3.8–5 μm basidiospores. This new species was collected in the tropical forest of Tabasco, Mexico. Description of the specimens, macroscopic images of the basidiomes, light microscopy, and scanning electron micrographs are provided. DNA extraction was carried out following a CTAB protocol. GeneBank and newly generated ITS, LSU, and Tef1α sequences were used in Maximum Likelihood and Bayesian Inference analyses. Phylogenetically, T. subreticulatum is related to T. deltaconcavum and forms a sister group from Clade 11 of the monophyletic genus Tulostoma. Comparisons with related sequenced and non-sequenced species are discussed. With this species, a total of 51 species of Tulostoma are known in Mexico.

Introduction

The members of the genus Tulostoma Pers (1794: 86). are characterized by their angiocarpic basidiomes formed by a globose spore-sac attached to a well-defined hollow stipe. The species of this genus are found in diverse habitats, from arid to temperate zones in all continents except Antarctica (Wright, 1987). Since the world taxonomic monograph of the genus Tulostoma by Wright (Op. cit.), who considered 139 species, more than 20 species of the genus have been described. Some of them were based exclusively on morphological traits, such as T. submembranaceum G. Moreno, C. Ochoa & J.E. Wright (1995: 117), T. tropicale Guzmán, Montoya & Bandala (1992: 114) and T. pseudopulchellum G. Moreno, Altés & J.E. Wright (1992: 481) from Mexico (Guzman et al. 1992, Moreno et al. 1992a, Moreno et al. 1995), T. matae Calonge & J. Carranza (2003: 38) from Costa Rica (Calonge & Carranza, 2003), T. lacrimisporum L. Fan & B. Liu (2005: 159) and T. verrucicapillitium L. Fan & B. Liu (2005: 160) from China (Fan & Liu, 2005), and T. irregulireticulatum Dourado-Barbosa, R.L. Oliveira, A.A. Lima, Baseia & R. Cruz (2023: 97) from Brazil (Dourado-Barbosa et al. 2023).

Materials and methods

Location description. The examined specimens were collected in a rock pit filled with organic matter and moss in the Agua Blanca State Park (PEAB) in October 2019 and kept in the herbarium. The PEAB is between 17°35’–38’ N and 92°25’–29’ W and ranges from 100 to 200 m ASL (Figure 1). The area comprises a surface area of 2,025 ha in Macuspana, Tabasco, Mexico (INEGI, 1986). Two main landforms can be seen in its northernmost part, which shares the karstic plain and the Uvala with the Sierra de Chiapas (INEGI, 1986; Castillo-Acosta & Zavala, 1996; Zarco-Espinosa et al. 2010). The climate is characterized by a warm, humid trend Af (m) w” (i) g, annual average temperatures ranging from 23 to 26°C, and an annual precipitation of 2,100–3,200 mm. Average yearly rainfall is divided into two seasons: rainy, from June to November, and a scarce rainy season from December to May (SEDESMA, 2000; INEGI, 1994). Agua Blanca belongs to the Grijalva-Usumacinta hydrological region (RH30), within the Grijalva-Villahermosa River basin and the Macuspana River sub-basin. The main river systems in the park are the Tepetitán, Puxcatán, Tulija, Maluco, and Chilapa. There are also underground runoff streams that come down from the mountains and form waterfalls and natural pools. The surface water networks are torrential dendritic and short drains that disappear into cave systems (Castillo-Acosta & Zavala, 1996). The 2,000 ha of high evergreen forest is dominated by over 30 m tall trees, covered in vines and epiphytic plants. The composition of arboreal species consists mainly of the following: canshán (Terminalia amazonia), ramión (Brosimun alicastrum), palo mulato (Bursera simaruba), guapaque (Dialum guianense), mahogany (Swietenia macrophylla), sapote mamey (Pouteria sapota), ceiba (Ceiba pentandra), buttonwood (Rinorea guatemalensis), among others. The park presents a high plant diversity, with approximately 1,950 species of vascular plants from 150 families, which encompasses 49% of the whole state’s plant diversity. Therefore, PEAB is one of the last remnants of natural vegetation in Tabasco State. Secondary vegetation includes crop waste, compost, grasslands, and hydrophilic vegetation in low-lying sites (Miranda & Hernández, 1963; Castillo-Acosta, 1995; Zarco-Espinosa et al. 2010). The soils developed from limestone rock weathering, creating a rendzina-type soil. This soil has a very thin film of deposition that is no more than 20 cm thick and forms on top of the limestone rock. This creates a dark-colored “A” horizon-type soil high in nutrients and organic matter. It also has a fine eutric lithosol layer, a thin middle layer, and internal drainage since the rocks are porous (INEGI, 2003).

Morphological Examination. The collected specimens were characterized macroscopically and microscopically based on Wright (1987). The color codes in parentheses are from Kornerup & Wanscher (1978). Photographs of basidiome details were obtained with a microscope Leica Z16 APO A (Wetzlar, Germany) and processed in the Leica Application Suite ver. 4.3.0 software (Leica Microsystems, 2023). Microscopic features were obtained by mounting basidiome and gleba fragments in KOH 10% and measured using the software Image Pro Plus 7.0 (Media Cybernetics, 2023).

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). All images
were captured using cameras on microscopes at the “Laboratorio de Microscopia y fotografía de la Biodiversidad”, from Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). The studied material is deposited in the macrofungi collection “Sala Psilocybe” of the National Herbarium (MEXU) of the Instituto de Biología de la Universidad Nacional Autónoma de México (IBUNAM), and the isotype is deposited in the herbarium of the Universidad Juárez Autónoma de Tabasco (UJAT).

**FIGURE 1.** Map of the Locality Agua Blanca State Park in Macuspana Municipality, Tabasco State, Mexico.

**DNA extraction, PCR, and sequencing.** Genomic DNA was extracted with a modified CTAB protocol (Doyle & Doyle, 1987) as follows: a small portion of the stipe was placed in a tube along a sterilized tungsten sphere, and then the tubes were frozen with liquid nitrogen and pulverized using a TissueLyserLt (QIAGEN). Immediately, 500 μL of CTAB and 2 μL of β-mercaptoethanol were added per sample, and the tubes were incubated at 65°C for 30 min at 300 rpm. Then, 500 μL of SEVAG (chloroform: isoamyl alcohol, 24:1) was added and mixed for 30 min at 85 rpm and room temperature. The mix was centrifuged for 10 min at 13,000 × g, the supernatant was transferred to a 1.5 mL tube, and 500 μL of isopropanol was added, gently mixed by inversion, and stored at -20 °C overnight. The mix was centrifuged for 10 min at 12,000 × g, and the supernatant was discarded. The remaining pellet was washed with 70% cold EtOH, dried in a vacuum centrifuge for 5 minutes, and resuspended in 50 μL of ultrapure water. 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 LSU), we used the ITS1F/ITS4B and LR0R/LR5 primer pairs (Gardes & Bruns 1993; White et al. 1990). PCR reactions were carried out with the PCR Mix 2x (5’BIO, Mexico) following the manufacturer’s instructions in a volume of 20 μL, with a total of 20 ng of gDNA per reaction, and using the thermal cycler conditions described by Schoch et al. (2012). For Tef1α, 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 in the “Laboratorio de Secuenciación Genómica de la Biodiversidad y de la Salud” of IBUNAM, with the same primers used in each PCR.

**Phylogenetic analyses.** The obtained sequences were assembled and curated by inspecting their chromatograms with Geneious Prime® 2023.2.1. The obtained sequences were deposited in the GenBank. Reference sequences from *Tulostoma* species were downloaded from the NCBI database, and some Lycoperdaceae (Larsson & Jeppson, 2008) as outgroups (Table 1). Sequences were aligned using the online version of MAFFT version 7 (Katoh et al. 2002, 2017; Katoh & Standley, 2013). The alignments were reviewed in MESQUITE (Maddison & Maddison, 2023), followed by
minor manual adjustments to ensure character homology among the taxa. The matrix consisted of 44 sequences and a total of 1,901 positions. Phylogenetic inferences were estimated using the Maximum Likelihood Method in the online server of IQTree (Trifinopoulos et al. 2016), and the best model was selected using ModelFinder (Kalyaanamoorthy et al. 2017) with 1,000 bootstrap resampling replicates. Bayesian analysis was executed in Mr. Bayes v.3.2.7 (Ronquist et al. 2012). The information block for the matrix included two simultaneous runs, four Monte Carlo chains, a temperature set at 0.2, and a sampling of 10 million generations (standard deviation ≤0.1) with trees sampled every 1,000 generations. The two simultaneous Bayesian runs continued until convergence parameters were met, and the standard deviation fell below 0.0001 after 10 million generations.

**TABLE 1.** Species, vouchers, localities, and GenBank accessions of the *Tulostoma* specimens used for the phylogenetic analysis. Double accession indicates the separation of ITS from LSU. Accession numbers with * indicate only ITS. Sequences obtained in this study are marked in bold.

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<th>Country of origin</th>
<th>Accession Number (NCBI)</th>
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TABLE 1. (Continued)

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Results

Phylogenetic analysis. Blast-N of the ITS region showed a similarity of 96% with *T. deltaconcavum* and 89% with *T. calcareum* Jeppson, Altés, G. Moreno & E. Larss. (2017: 46). The full ITS and LSU showed a similarity of 90.66% with *T. calcareum* and 89.71% with *T. melanocyclum* Bres. (1904: 415). The partial Tef1α showed a similarity of 88% with Tulostoma sp. 3 (collection MJ4935 in Jeppson et al. 2017). From the 1,901 positions, 1,434 are conserved, and 459 are variable, of which 363 are informative and 95 are singletons. Maximum Likelihood and Bayesian Inference analyses showed an identical topology (Figure 2) and placed our collection in the monophyletic genus Tulostoma, close to *T. deltaconcavum* (BS/BPP: 100/1). Both species form a sister clade from Clade 11 (Jeppson et al. 2017) of the genus with high support (94/1), here provisionally named Clade 12. The analysis suggests that Tulostoma subreticulatum Hern.-Nav. & Cappello-Gar is a new species for science.
**FIGURE 2.** Phylogeny based on the nrITS, LSU, and Tef1α sequence data. Maximum likelihood and Bayesian analyses. For each node, the following values are provided: maximum likelihood bootstrap (0-100) / and posterior confidence (p-value: 0-1). The scale bar represents the expected number of nucleotide substitutions per site. The new species, *Tulostoma subreticulatum*, is shown in bold red.

**Taxonomy**

*Tulostoma subreticulatum* Hern.-Nav. & Cappello-Gar. *sp. nov.*

(FIGURES 3 & 4)


**MycoBank:** #852431

**GeneBank accession numbers:** OR539673 (ITS-LSU), PP375809 (Tef1α)

**Etymology:** The name refers to the subreticulate appearance of the basidiospores.

**Diagnosis:** basidiome up to 48 mm high, stipe 24 × 1.5–2.5 mm, reddish-brown, with a mycelial bulb with hyphae encrusted with debris, spore-sac 5–11 mm diam. × 5–8 mm height, circular mouth, verrucose exoperidium, reddish brown endoperidium, subreticulate basidiospores, hyaline capillitium with visible lumen, slightly swollen at the yellowish septa.
Description: Basidiome up to 48 mm high, stipitated. Spore-sac subglobose, 5–11 mm diam. × 5–8 mm height. Mouth circular to elliptical, less than 1 mm diam. Exoperidium membranous, brown (E6E) to grayish brown (6E4), verrucose, composed of angular to cubic verrucae up to 200 μm high, easily removable. Persistent in the base of the spore-sac, leaving a sub-reticular pattern in the upper part. Endoperidium reddish brown (8D6), mottled with some verrucae and verrucae scars. Gleba light ferruginous (6B6–6A7). Socket conspicuous, fibrillose, separated from the stem. Stipe, mostly less than 24 mm, but up to 40 mm × 1.5–2.5 mm, reddish brown (8D6), very thin and fragile, striated in the base to slightly squamosal in the upper third, with a conspicuous basal bulb with hyphae strongly mixed with grains of sand and debris, as the rhizomorphs.

Verrucae formed by irregular and pigmented pseudo parenchymatous hyphae, up to 26 μm in the longest portion and up to 14 μm in the shortest, with a cracked to granulose appearance, with cell walls up to 1 μm. Endoperidium is formed by short and wavy hyaline hyphae, 3–6 μm diam., septated, and slightly swollen at the yellowish septa in KOH. Stipe composed of hyphae 19–33 μm in length from one septum to another; 6–10 diam. The external cells are shorter, darker, and swollen; the internal hyphae are sub-hyaline to hyaline and slimmer. Basidia not observed. Basidiospores globose to occasionally subglobose, 3.5–4.5 × 4–5 μm, including ornamentation, yellowish, verrucose to subreticulated. Under SEM, the ornamentation is formed by anastomosed elements, forming variable patterns and an incomplete reticulum. Capillitium hyphae hyaline to slightly yellowish, 3–5 μm wide, lumen visible, with yellowish to light brown pigmented septa, slightly swollen up to 6 μm.
**Habit and habitat:** growing gregariously in a tropical forest as saprobe, in a rock pit and in soil, with abundant organic matter and moss.

**Notes:** This is a distinct species due to the combination of characteristics of small to medium-sized basidiomes, tubular stoma, verrucose exoperidium, reddish-brown endoperidium, and small, subreticulate basidiospores. The ornamentation of basidiospores is conformed by anastomosed elements forming irregular patterns that tend to form a subreticulum, visible under L.M. with a good oil-immersion lens (Figure 4a). According to Wright (1987), subreticulated basidiospores can be formed by anastomosed verrucae or spines. Some examples are *T. subsquamosum* Long & S. Ahmad (1947: 241) with fused spines, *T. cyclophorum* Lloyd (1906:25) with low fused verrucae, or *T. purpustii* (Henn. 1898: 274), described as “basidiospores spores with numerous appressed verrucae fusing in rib-like structures” under the LM and “anastomosed crest, which exhibits notorious likeness to a subreticulum”. A similar case is *T. dumeticola*, which is described as having anastomosed finger-like spines that appear almost reticulated. On the other hand, Wright (op. cit.) describes “asperulated”, some species with asperulated basidiospores both under LM and SEM; for example, *T. xerophilum* Long (1946: 85) or *T. albicans* White (1901:428). In these cases, the basidiospores are almost smooth at 100× with low and irregular verrucae that can be seen with a good oil immersion lens or phase contrast microscopy. Under SEM, both species have irregular verrucae and truly asperulated spores. Other species are described as asperulate at L.M. but with different kinds of ornamentation at SEM; for example, *T. nanum* (Pat.) J.E. Wright (1987: 160) has asperulate basidiospores at L.M. but with minute verrucae on SEM. In *T. macrocephalum* Long (1944: 337), the ornamentation is formed by low-crested verrucae on SEM. Based on ITS nrDNA, *T. subreticulatum* is close to *T. deltaconcavum*, but the latter has non-verrucose exoperidium, composed of polymorphous hyphae, yellowish, branched, and septate, without pseudoparenchymatous hyphae. In addition, the basidiospores of *T. deltaconcavum* present concave, triangular spines, not subreticulated. (Lima et al. 2023). Both species group with high support as a sister group of Clade 11, which is composed of eight species with circular ostioles, reddish stipes, and coarsely ornamented spores: *T. calcareum*, *T. subsquamosum*, *T. ahmadii*, *T. squamosum*, *T. dominguenziae*, and *rufum* Lloyd (1906: 18), *T. melanocyclum*, and Tulostoma sp. 22 (an undescribed species from South America). All of these species mentioned above present stouter basidiomes than *T. subreticulatum*. In addition, except for *T. subsquamosum*, all present echinulate basidiospores with independent spines, not subreticulated, and, in *T. subsquamosum*, ornamentation is conformed by spines fused at the apex, forming crests. On the other hand, *T. subsquamosum* and *T. rufum* present hyphal exoperidium and mycosclereids but no verrucae (Jeppson et al. 2017; Wright, 1987). *T. melanocyclum* is characterized by the noticeable dark peristome and hyphal exoperidium but lacks mycosclereids. (Wright et al. 1987). The rest of the species in the clade 11 present verrucose exoperidium; however, they differ in the hyphal structure of the verrucae, a characteristic that is only sometimes properly measured and described. *T. ahmadii*, from Pakistan, presents light olive brown verrucae, composed of subglobose to elongated pseudo parenchymatous hyphae, 7–10 × 2–4 μm, irregularly arranged. *T. calcareum*, from Europe, was described as having a deciduous hyphal-verrucose exoperidium, but the hyphal structure of the verrucae was not described (Jeppson et al. 2017). *T. dominguenziae* presents reddish-brown warts conformed by pigmented pseudoparenchymatous hyphae up to 250 × 11 μm (Hernández-Caffort et al. 2011). In *T. squamosum*, the verrucae are composed of dark sphaerocystis (Jeppson et al. 2017; Esqueda et al. 2004); however, the size is not specified. Wright (1987) considered *T. squamosum*, *T. mussooriense* Henn. (1901: 337), and *T. verrucosum* Morgan (1890: 164) as independent species. In the latter, Wright (op. cit.) described vesicular hyphae freely arranged in chains (15–30 × 6–11 μm). These three taxa were considered synonymous by Moreno et al. (1992b), who reported spherocysts or chains of short subglobose to subcylindrical hyphae in the warts of the exoperidium of the type materials of the three species. Exact measurements were not specified, but based on the images presented and their scales, the largest are ~10 × 16 μm diam.

**Discussion**

Some other unsequenced species also present verrucose exoperidium but differ in important aspects. *T. dennisii* J.E. Wright (1987: 90) presents white to cinereous endoperidium, mycosclereids in the endoperidium, and larger basidiospores (5.5)6.8–7.8(8.5) μm, irregularly echinulate, with anastomosed columns. This species is known from Venezuela and Peru. On the other hand, *T. pusillum* Berk. (1842: 157), presents ochraceous to cinereus endoperidium, stipe up to 65 × 3 mm, basidiospores 6–7.8 μm with noticeable coalescent spines of pyramidal aspect. This species is known from the Philippines, Cuba, and Venezuela (Berkeley, 1842; Wright, 1987). From the known species in Mexico, *T. exasperatum* presents conical to pyramidal verrucae in the exoperidium, fibrillose ostiole, cream color, and truly
reticulated basidiospores with membranes as “wings”. In addition, the hyphae from the verrucae are described as “puzzle-like” with short, interwined, angulose, and pigmented globose to cystidioid terminal hyphae (Wright, 1987; Hernández-Navarro, 2023). The sequencing of specimens determined as T. exasperatum from Thailand demonstrated the autonomy of this species from Clade 11 (Paloí et al. 2023). Furthermore, the sequencing of Brazilian specimens of T. exasperatum differed from those in Thailand, suggesting a new species, T. paratyense (Cabral et al. 2023).

Another unsequenced similar species is T. dumeticola. The Mexican specimens of T. dumeticola from Veracruz also present irregularly subreticulated spores. Still, this species presents a profusely verrucose endoperidium, a smooth, light grayish endoperidium, longer and thicker stipes (35–70 × 2–3 mm), and bigger echinulate to subreticulated basidiospores 4.8–6.4 (~7.2) μm (Guzmán et al. 1992). Wright (1987) described mycosclereids 7–16.8 μm and thick walls for the Brazilian and Uruguayan materials, including the holotype of T. dumeticola; however, Guzmán et al. (1992) described “subglobose elements of dark orange-brown color, 7.2–21.6 μm in diameter” for the Mexican material. Another unsequenced species described from Mexico is T. tropicale; however, this species presents a membranous sub-verrucose exoperidium, a purplish gray endoperidium, and verrucose basidiospores 4–4.8 (~5.6) × (3.2) 4–4.8 μm, with verrucae lower than 0.8 μm. Also, the exoperidium’s verrucae are formed by dark, straight, clamped hyphae, 1.8–5.6 μm in diameter (Guzmán et al. 1992). The authors remarked on the affinity of the taxon with T. brumale Pers. (1794: 86) and T. similans Lloyd (1906: 18), members of Clade 10, but they did not provide a SEM image of the basidiospores. Based on the description and association with the taxa above, it seems to be a non-closely related species to the one presented here. Unfortunately, none of the T. dumeticola and T. tropicale from Mexico could be analyzed since the collections are missing from the XAL herbarium.

Tulostoma subreticulatum is phylogenetically related to T. deltaconcavum from Brazil, and both form a sister group of Clade 11 (Jeppson et al. 2017). All the species in this clade are European or Asiatic, except T. domiguezieae from Argentina and T. rufum from the USA. Since T. subreticulatum differs by at least 10% from the closest member of Clade 11, this might indicate a different new clade within the genus. Despite T. deltaconcavum only presenting ITS sequences, the three-gene phylogeny strongly supports its relationship with T. subreticulatum in an independent clade, here provisionally named Clade 12 (Figure 2).

It has been pointed out that Wright’s (1987) infrageneric ranks, based only on morphology, do not match up with the phylogenetic relationships based on the three-gene phylogeny proposed by Jeppson et al. (2017). Despite being the most comprehensive phylogenetic study to date, some of the clades were not entirely supported (e.g., Clades 2, 4, and 5) since some of the sequenced materials, including holotypes, are represented only by partial ITS sequences (e.g., T. xerophilum Long (1946: 85), T. macrocephallum Long (1944: 337). Despite this, the partial sequences of the type materials could help assign neotypes. Still, the infrageneric classification of Tulostoma is a matter of research since ~75% of the named species considered by Wright (1987) are unsequenced, and there is evident high cryptic diversity within the genus. Including sequences from collections from other localities and more molecular markers, such as partial β-tubulin II (TUB2), γ-actin (ACT), and RNA polymerase II large subunits 1 and 2 (RPB1/2), could improve the topology and current understanding of the genus.

Conclusion

Tulostoma subreticulatum is a distinct species based on morphology and nucleotide sequences. This taxon makes a total of 51 species of Tulostoma known from Mexico. This is the second species to be described from Mexico with nucleotide sequences.

Key to Tulostoma species with tubular stoma and verrucose exoperidum

1. Sphaerocyst present in the endoperidium ........................................................................................................................................ 2
   - Sphaerocyst absent in the endoperidium .................................................................................................................................... 3

2. Sphaerocyst abundant in endoperidium; echinulate basidiospores 4.7–6.5 μm not subreticulated ................................................ T. squamosum
   - Sphaerocyst rare in the endoperidium; sub reticulated basidiospores 4.6–6.1 μm ................................................................. T. subsquamosum

3. Uncolored endoperidium ......................................................................................................................................................... 4
   - Colored endoperidium ......................................................................................................................................................... 7

4. Mycosclereids absent in the endoperidium .......................................................................................................................... 5

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- Mycosclereids present in the white to cinereous endoperidium; basidiospores (5.5) 6.8 – 7.8 (8.5) μm, irregularly echinulate, with anastomosed columns ...........................................................................................................................................................................

5. Exoperidium hyphal-verrucose, stipe slender 20–50 × 2–3 mm, basidiospores 4.0–6.0 μm .................................................................................. T. calcareum
- Stouter stipes with larger basidiospores .................................................................................................................................................................................................

6. Light brown verrucae, creamy to grayish white endoperidium, stipe 20–35 × 4–6 mm basidiospores 7–9 × 6.8 μm, echinulate......
- Reddish brownish verrucae conformed by pseudo parenchymatous cells up to 250 μm in length, basidiospores (5.73 × 6.25(8.7) μm ..... T. loonbanglaense

7. Ovoid basidiospores 6–8 × 4–5 μm; exoperidium with dark brown verrucae, endoperidium cinnamon brown ............ T. matae
- Basidiospores globose.........................................................................................................................................................................................................................

8. Basidiospores subreticulated ............................................................................................................................................................................................... T. dominguenziae
- Basidiospores not subreticulated ......................................................................................................................................................................................................

9. Spore-sac 5–11 mm diam, light brown verrucae, reddish brown endoperidium, stipe up to 30 mm, basidiospores 3.5–5 μm, sub reticulated ................................................................................................................................................................................................. T. subreticulatum
- Spore sac 9–13 mm diam, dark brown verrucae, brown endoperidium, stipe 35–50 × 2–2.5 mm, basidiospores 5.4–7.2 μm, sub reticulated ............................................................................................................................................................................................................. T. dumeticola

10. Basidiospores verrucose 4–4.8 (5.6) × (3.2) 4–4.8 μm, with low verrucae up to 0.8 μm; exoperidium sub verrucose, purplish gray endoperidium ...................................................................................................................................................................................................... T. tropicale
- Basidiospores echinulate ....................................................................................................................................................................................................

11. Light olive brown verrucae, pinkish endoperidium, echinate basidiospores (6)7.5–9.4(10.3) × (4)6.3–8.2(9.4) μm .... T. ahmadii
11b. Dark brown verrucae, ochraceous to cinereous endoperidium, stipe 65 × 3mm, basidiospores 6–7.8 μm with notable pyramidal spines ................................................................................................................................................................................................................ T. pusillum

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