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Taxonomy and systematics

Diversity, morphological variability, and distribution of tulostomataceous fungi (Agaricomycetes) in Sonora, Mexico

Diversidad, variabilidad morfológica y distribución de hongos tulostomatáceos (Agaricomycetes) en Sonora, México

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Abstract

Recent mycological studies in Sonora, Mexico have mainly focused on tulostomataceous fungi, a group of gasteroid Agaricomycetes with a spore-sac containing pulverulent gleba and a well-defined, hollow stipe. The aim of the present study was to analyze the diversity, morphological variability, and distribution of tulostomateceous fungi, through examining the specimens deposited in the Sonora State University herbarium over 29 years of sampling. Based on 1,266 basidiomes in 621 collections from 75 locations, 23 municipalities, and 16 vegetation types, a total of 7 genera and 37 species were identified. All genera are monospecific in Sonora except *Tulostoma*, with 31 defined species. Several collections remain undetermined since their morphological traits are intermediate or do not match described species. The distribution of tulostomataceous fungi was related to altitude, precipitation, and climate. Further molecular analysis of undetermined specimens must be performed in order to define the real number of species in this fungal group and to understand the boundaries among closely related genera and species.

Keywords: Basidiomycota; Gasteroid fungi; Diversity; Chorology

Resumen

Los estudios micológicos recientes en Sonora, México, se han enfocado principalmente en hongos tulostomatáceos, un grupo de Agaricomycetes gasteroides con basidiomas compuestos por un saco esporífero con gleba pulverulenta y un estípite hueco bien definido. El objetivo del presente estudio fue analizar la diversidad, variabilidad morfológica y distribución de hongos tulostomatáceos, con los especímenes depositados en el herbario de la Universidad Estatal de

Sonora por 29 años. Con base en 1,266 basidiomas de 621 recolecciones provenientes de 75 localidades, 23 municipios y 16 tipos de vegetación, se determinaron 7 géneros y 37 especies. Todos los géneros son monoespecíficos en Sonora, excepto *Tulostoma* con 31 especies definidas. Varias recolecciones permanecen indeterminadas por sus características morfológicas intermedias o no coinciden con las especies descritas. La distribución de los hongos tulostomataceos se relacionó con altitud, precipitación y clima. Deberán realizarse análisis moleculares de los especímenes indeterminados para definir el número real de taxones y entender los límites entre géneros y especies afines.

Palabras clave: Basidiomycota; Hongos gasteroides; Diversidad; Corología

Introduction

Mexico is a country with a high biodiversity, including fungal diversity. It ranks thirteenth in the world in territorial surface area (1,964,375 km²) yet fifth in biodiversity. Of the 32 federal entities of Mexico, Sonora is the second largest, with an area of 179,355 km², which is larger than the smallest 106 countries in the world. In regard to number of fungal species, Sonora ranks fifth in Mexico, with 658 recorded; of these, 461 are Basidiomycetes (Aguirre-Acosta et al., 2014). The most studied groups in Sonora are gasteroid and sequestrate Agaricomycetes (GSA), with more than 120 cited species. Of these, 37 correspond with tulostomataceous stalked-puffballs (TSPs), which are the most diverse and abundant group in Sonora (Esqueda, Pérez-Silva, Herrera, Altés et al., 1998; Esqueda et al., 2004, 2010; Hernández-Navarro et al., 2015, 2017, 2018; Piña-Páez et al., 2010).

The main morphological traits of TSPs are the presence of a spore-sac with pulverulent gleba and a well-defined, hollow stipe, with a volva or a volvoid structure; sometimes, rhizomorphs are present. The genera are mainly differentiated based on the continuity of the stipe, peridium layers, gleba type, presence and type of capillitium, dehiscence, and spore ornamentation. Notably, TSPs are especially abundant, diverse, and predominant in arid and semiarid zones, although some species grow exclusively in subtropical, tropical, or temperate regions. All species present terrestrial habits, with the remarkable exception of *T. exasperatum* Mont., which is lignicolous. Some studies have provided *in situ* information on fructification sites, which can help to understand the species autecology, although this task is still far from complete (Cortez et al., 2009; Esqueda et al., 2000; Hernández-Navarro et al., 2015; Wright, 1987).

Originally, TSPs were classified as belonging to the family Tulostomataceae, which consisted of 8 genera classified in 3 tribes: Battarreae (*Battarreia*, *Battarreoides*), Phellorinae (*Chlamydopus*, *Dictyocephalos*, *Phellorinia*), and Tulostomatinae (*Queletia*, *Schizostoma*, *Tulostoma*) (Long, 1946; Long & Stouffer, 1946; Wright, 1987). Although several species have been described for each

genus, most genera are monotypic or have few taxa, except for *Tulostoma* which, according to the world monograph of Wright (1987), is composed of 139 spp. Currently, more than 155 species have been described for the genus (www.speciesfungorum.org/names/fundic.asp).

Considering molecular data, several changes have occurred in fungal classification, including the invalidation of the class Gasteromycetes and its segregation into several orders such as Agaricales, Boletales, Russulales, Geastrales, and Phallales. Tulostomataceae was also segregated into 2 families, Phelloriniaceae (including *Phellorinia* and *Dictyocephalos*) and Agaricaceae (the rest of the genera). Agaricaceae has traditionally been one of the most studied and best recognized fungal families. However, it is currently considered a heterogeneous family with diverse growth forms such as agaricoid, sequestrate, and gasteroid. Despite the lack of molecular information of TSPs and due their distinctive morphological traits, some authors have suggested that they represent a monophyletic lineage, independent from Agaricaceae in the agaricoid clade of Agaricales (Gube, 2009; Martin et al., 2000; Matheny et al., 2006; Vellinga, 2004).

Up until 2018, 7 of the 8 known genera of TSPs have been reported in Sonora and a total of 37 species. Some of these species are cosmopolitan or with a wide distribution, but a few are also infrequent (*Battarreia*), rare (*Chlamydopus*, *Dictyocephalos*), or extremely rare such as *Tulostoma gracilipes* and *T. portoricense*, which were cited for the second time worldwide from Sonora (Esqueda, Pérez-Silva, Herrera, Altés et al., 1998; Esqueda, Pérez-Silva, Herrera & Moreno, 1998; Esqueda et al., 2000; Piña-Páez et al., 2010). The aim of the present study was to analyze the diversity, morphological variability, and distribution of TSPs, through examining the specimens deposited in the herbarium of the Sonora State University over 29 years of sampling.

Materials and methods

All TSPs in the macromycetes collection of the Sonora State University herbarium (UES - for its initials in Spanish) were characterized macro- and

microscopically according to the methods previously described by Hernández-Navarro et al. (2015, 2017), including scanning electron microscopy. For the microscopic characterization, temporary preparations of the glebal portions were mounted with 10% KOH, which is recommended by Wright (1987) to rehydrate the material, allowing it to recover its shape and making the ornamentation more easily discernible without altering the color. We measured at least 20 spores, capillitia, septa, and elaters per collection. The recognition of morphological species was based on the combination-of-characteristics approach proposed by Wright (1987). Finally, we gathered the annotated information from the collections: location, municipality, altitude, climate, soil, and vegetation type (Table 1).

Data analysis was done in the statistical environment R version 3.0.3 (www.R-project.org) using the vegan (Oksanen et al., 2019) and cluster packages (Maechler et al., 2019). Canonical Correspondence Analysis (CCA) was used to elucidate the relationships between TSPs and environmental conditions, and significances were tested by permutational multivariate analysis of variance (PerMANOVA) ($p < 0.01$). Hierarchical cluster analysis (HCA) using Ward's method was used to group TSPs, taking into account environmental conditions (Ward, 1963).

Results

Based on 1,266 specimens in 621 collections, 37 species belonging to 7 genera of TSPs were determined (Table 2; Fig. 1). Tribe Battarreae was represented by both of its genera, *Battarrea* and *Battarreoides* with more collections of *Battarreoides diguetii* than *Battarrea phalloides*. Tribe Phellorinae was the least abundant group. There was only one specimen of *Dictyocephalos attenuatus* found in the “El Pinacate” and “Gran Desierto de Altar” Biosphere Reserves. *Chlamydopus meyenianus* was also a rare species with only 2 specimens. *Phellorinia* had 6 specimens, one per collection. Tribe Tulostomatinae was the most abundant and diverse, composed of 2 genera, *Tulostoma* and *Schizostoma*. The latter genus had only one species (*S. laceratum*) in Sonora. On the other hand, *Tulostoma* was the most abundant and diverse genus, with 1,090 specimens in 523 collections corresponding to 31 species. Several *Tulostoma* species were especially abundant, such as *T. fimbriatum* (53 collections, 146 specimens), *T. xerophilum* (44 collections, 98 specimens), *T. albicans* (48 collections, 88 specimens), *T. chudaei* (45 collections, 95 specimens), and *T. cretaceum* (32 collections, 96 specimens) (Fig. 2). Some spore ornamentation under SEM of *Tulostoma* species are shown in figures 3 and 4.

Table 1
Abiotic characteristics, type of vegetation, and coordinates by locality.

Municipality/ Locality	A	V	C	P	S	N	W
Álamos							
1 El Aguaje	734	TTF	SDSW	673	Re	26.97	108.96
2 El Sabinito	364	TTF	SDW	673	Li	27.00	108.80
3 El Encinal	1494	OF	SDT	673	Re	26.97	108.98
4 Km 7.5 Álamos-Guirocoba	372	TTF	SDW	673	Ca	26.95	108.94
5 Palo Injerto	415	TTF	TSW	673	Li	27.05	108.73
Bacoachi							
6 Km 146 Mazocahui-Cananea	1046	AL	SDT	421	Ca	30.65	109.97
7 Km 164 Mazocahui-Cananea	1162	G	SDT	421	Ca	30.74	110.01
Baviácora							
8 La Cieneguita	1012	SS	SDSW	502	Ca	29.54	110.01
9 Mazocahui	507	MDS	DW	502	Ca	29.54	110.12
Benjamín Hill							
10 Las Ánimas	798	MDS	VDSW	314	Li	30.21	111.32
Caborca							
11 Rancho La Chula	257	AL	VDSW	100	Ye	30.70	112.25
Carbó							
12 San Luis	500	M	VDSW	192	Ye	29.56	111.08

Table 1
Continued

Municipality/ Locality	A	V	C	P	S	N	W
Cumpas							
13 El Mezquital	820	IG	DSW	474	Ca	29.96	109.64
14 Km 8 Ajos-Bavispe	879	CG	DSW	474	Ca	29.98	109.66
15 Km 8.5, Ajos-Bavispe	882	CG	DSW	474	Ca	29.98	109.66
16 La Selva	884	M	SDSW	474	Re	29.96	109.62
Fronteras							
17 El Frijolito	2370	POF	TSW	687	Re	30.94	109.96
18 El manzano	2296	OF	TSW	687	Re	30.93	109.97
19 La Sal	2028	OF	TSW	687	Re	30.96	109.95
General Plutarco Elías Calles							
20 Cráter El Celaya	264	MDS	VDSW	191	Ye	31.99	113.46
21 Cráter El Colorado	204	SCS	VDSW	191	Ye	31.92	113.31
22 Cráter El Elegante	167	SCS	VDSW	191	Ye	31.86	113.38
23 Sierra Los Tanques	488	MDS	VDSW	191	Ye	31.77	113.01
24 El Papalote	319	M	VDSW	191	Re	31.93	113.03
25 San Juanico	193	SCS	VDSW	191	Re	31.83	113.34
26 WPT del Colorado	197	HV	VDSW	191	Ye	31.89	113.29
Guaymas							
27 Cañón de Nacapule	173	SCS	VDW	328	Li	28.02	111.05
28 Ejido Francisco Villa	168	SCS	VDW	219	Ye	28.11	111.02
29 Maytorena	146	SCS	VDW	328	So	28.23	110.81
Hermosillo							
30 Calle 0 La Costa de Hermosillo	96	MDS	VDW	157	Ye	28.88	111.35
31 Centro Ecológico de Sonora	262	M	VDW	342	Re	29.02	110.95
32 CIAD	255	UZ	VDW	342	Re	29.13	110.91
33 El Crucero La Costa de Hermosillo	21	MDS	VDSW	169	So	28.83	111.72
34 El Apache	36	M	VDW	123	So	28.32	111.24
35 El Papalote	299	M	VDSW	342	Re	29.22	111.04
36 Km 90 Hermosillo-Bahía de Kino	12	SCS	VDSW	168	So	28.83	111.77
37 La Milla UNISON	202	UZ	VDW	342	Ye	29.08	110.97
38 La Pintada	218	SCS	VDW	342	Re	28.56	111.01
39 La Primavera	163	MDS	VDW	280	Re	28.80	111.15
40 La Tijerita	296	M	VDW	342	Re	29.17	110.92
41 La Victoria	236	AL	VDW	342	Ye	29.12	110.89
42 Rancho Las Palomas	216	M	DSW	292	Xe	28.99	110.46
Huásabas							
43 Sierra La Madera	1304	SS	SDT	438	Re	29.90	109.46
Huatabampo							
44 Huatabampito	11	AL	VDW	326	Ve	26.84	109.64
La Colorada							
45 Km 31 Hermosillo-Yécora	290	M	VDSW	343	Ye	28.85	110.71
46 Km 40 Hermosillo-Yécora	335	M	VDSW	343	Ye	28.82	110.63
47 Km 100 Hermosillo-Yécora	472	TS	DSW	489	Re	28.62	110.12
Opodepe							
48 Tuape	670	MDS	VDSW	354	Xe	30.05	111.01

Table 1
Continued

Municipality/ Locality	A	V	C	P	S	N	W
Pitiquito							
49 Ejido 15 de Mayo	269	MDS	VDSW	90	Re	29.79	112.53
50 Entrada a Puerto Libertad	9	MDS	VDSW	90	Re	29.90	112.66
51 Km 158, 36 N a Puerto Libertad	28	MDS	VDSW	90	Re	29.88	112.64
52 Las Dunas, Punta Cirio	40	MDS	VDSW	90	Re	29.85	112.64
53 Km 115, 36 N a Puerto Libertad	112	MDS	VDSW	90	Re	29.64	112.30
54 Km 125, 36 N a Puerto Libertad	127	MDS	VDSW	90	Re	29.68	112.38
55 Rancho Punta Cirio	212	MDS	VDSW	90	Re	29.82	112.57
Puerto Peñasco							
56 Ejido Los Norteños	119	SDV	VDSW	137	Re	31.66	113.33
57 Ejido Punta Peñasco	163	SDV	VDSW	137	Re	31.76	113.27
58 Sierra Blanca	69	MDS	VDSW	137	Re	31.52	113.42
Rayón							
59 Rancho La Granada	640	MDS	DSW	464	Re	29.69	110.50
San Javier							
60 Km 137 Hermosillo-Yécora	496	TDF	SDSW	684	Re	28.58	109.78
61 Km 151 Hermosillo-Yécora	720	TDF	SDSW	684	Re	28.58	109.68
62 Km 3.5 a San Javier	760	TDF	SDSW	684	Re	28.58	109.75
San Luis Río Colorado							
63 Cerro Lava / Microondas	246	SDV	VDSW	54	Re	32.05	113.56
64 San Luis Río Colorado, ciudad	42	UZ	VDW	78	Re	32.45	114.76
Soyopa							
65 Km 162 Hermosillo-Yécora	260	TDF	SDW	576	Li	28.56	109.60
66 Km 162.5 Hermosillo-Yécora	287	TDF	SDW	576	Li	28.56	109.60
67 Km 163 Hermosillo-Yécora	278	TDF	SDW	576	Li	28.57	109.60
68 Río Yaqui	213	TTF	SDW	576	Lu	28.57	109.55
Ures							
69 Km 72 Hermosillo-Mazocahui	1378	M	DW	371	Xe	29.45	110.29
70 Sierra Huérana, Bosque de encino	886	OF	SDT	478	Ca	29.10	110.20
71 Sierra Huérana, Matorral subtropical	599	SS	SDSW	393	Li	29.10	110.25
72 Sierra Huérana, Mezquital	145	M	SDSW	478	Xe	29.21	110.15
Yécora							
73 Km 200.5 Hermosillo-Yécora	915	TDF	SDSW	651	Ca	28.47	109.34
74 Km 205 Hermosillo-Yécora	900	TDF	TSW	651	Ca	28.46	109.31
75 Km 258 Hermosillo-Yécora	1476	OF	TSW	1057	Li	28.37	109.07

Headers: (A) Altitude (m asl); (V) vegetation; (C) climate; (P) precipitation (mm); (S) soil type; (N) north latitude; (W) west longitude. Vegetation types: (AL) agricultural lands; (CG) cultivated grassland; (G) grassland; (HV) halophytic vegetation; (IG) induced grasslands; (M) mezquital; (MDS) microphyllous desert scrub; (OF) oak forest; (POF) pine oak forest; (SDV) sandy desert vegetation; (SCS) sarcocaulle scrub; (SS) subtropical scrub; (TS) thorn scrub; (TDF) tropical deciduous forest; (TTF) tropical thorn forest; (UZ) urban zone. Climate types: (DSW) dry semiwarm; (DW) dry warm; (SDSW) semidry semiwarm; (SDT) semidry temperate; (SDW) semidry warm; (TSW) temperate subwet; (VDSW) very dry semiwarm; (VDW) very dry warm. Soil type: (Ca) Cambisol; (Li) Lithosol; (Lu) Luvisol; (Re) Regosol; (So) Solonchak; (Ve) Vertisol; (Xe) Xerosol; (Ye) Yermosol.

Table 2

Distribution of species by locality.

Species	Locality
<i>Battarrea phalloides</i> (Dicks.) Pers.	2, 11, 28, 31, 33, 37, 38
<i>Battarreoides diguetii</i> (Pat. & Har.) R. Heim & T. Herrera	8, 11, 27, 28, 29, 32, 34, 38, 39, 47, 48, 56, 58, 72
<i>Chlamydopus meyenianus</i> (Klotzsch) Lloyd	49, 51, 53
<i>Dictyocephalos attenuatus</i> (Peck) Long & Plunkett	22
<i>Phellorinia herculeana</i> (Pers.) Kreisel	23, 24, 47, 72
<i>Schizostoma laceratum</i> (Ehrenb. ex Fr.) Lév.	21, 22, 23, 23, 24, 29, 35, 39, 41, 46, 47, 48, 51, 53, 56, 57, 63
<i>Tulostoma albicans</i> V.S. White	10, 11, 12, 13, 14, 23, 27, 29, 35, 39, 47, 48, 53, 70, 71, 74
<i>T. amnicola</i> Long & S. Ahmad	1, 10, 22, 45, 46, 47
<i>T. australianum</i> Lloyd	24, 52
<i>T. beccarianum</i> Bres.	10, 39, 47, 65
<i>T. chudaei</i> Pat.	12, 13, 15, 22, 24, 29, 38, 39, 45, 46, 47, 48, 52, 56, 59, 60, 63, 72, 72
<i>T. cretaceum</i> Long	8, 11, 22, 24, 25, 35, 36, 39, 41, 44, 48, 49, 53, 54, 56, 57, 58, 62, 63, 64
<i>T. cyclophorum</i> Lloyd	31
<i>T. dumeticola</i> Long	3, 60, 61
<i>T. fimbriatum</i> Fr.	5, 6, 10, 12, 13, 16, 21, 24, 26, 28, 31, 35, 38, 40, 46, 47, 48, 65, 67, 68, 75
<i>T. floridanum</i> Lloyd	4, 8, 60, 70
<i>T. gracilipes</i> J.E. Wright	47, 72
<i>T. involucreatum</i> Long	8, 12, 27, 38, 43, 45, 47, 66, 72
<i>T. leiosporum</i> R.E. Fr.	14, 20, 22, 23, 24, 29, 39, 45, 47, 48, 57, 60, 63, 65, 66, 68
<i>T. longii</i> Lloyd	47
<i>T. macrocephalum</i> Long	22, 34
<i>T. macrosporum</i> G. Cunn.	7, 10, 14, 29, 33, 38, 41, 45, 47, 48, 72
<i>T. melanocyclum</i> Bres.	16, 17, 18, 19, 53, 60
<i>T. membranaceum</i> Long & S. Ahmad	47
<i>T. mohavei</i> Lloyd	23, 41, 45, 47
<i>T. nanum</i> (Pat.) J.E. Wright	10, 11, 12, 22, 23, 25, 28, 30, 38, 45, 46, 47, 48, 54, 56, 60, 61, 66, 69, 73
<i>T. obesum</i> Cooke & Ellis	10, 20, 22, 24, 25, 34, 38, 41, 50, 55, 56, 57, 58, 63
<i>T. portoricense</i> J.E. Wright	7
<i>T. pulchellum</i> Sacc.	14, 45, 46, 47
<i>T. pygmaeum</i> Lloyd	12, 13, 16, 23, 25, 28, 29, 39, 47, 48, 66, 71, 72, 74
<i>T. rufescens</i> Hern.-Nav. & Esqueda	43
<i>T. squamosum</i> (J.F. Gmel.) Pers.	60
<i>T. striatum</i> G. Cunn.	13, 31
<i>T. subfuscum</i> V.S. White	8, 13, 31, 47, 63
<i>T. submembranaceum</i> G. Moreno, C. Ochoa & J.E. Wright	18, 29, 42, 66, 68
<i>T. wrightii</i> Berk.	13, 63
<i>T. xerophilum</i> Long	9, 10, 11, 24, 38, 46, 47, 48, 65, 66, 72

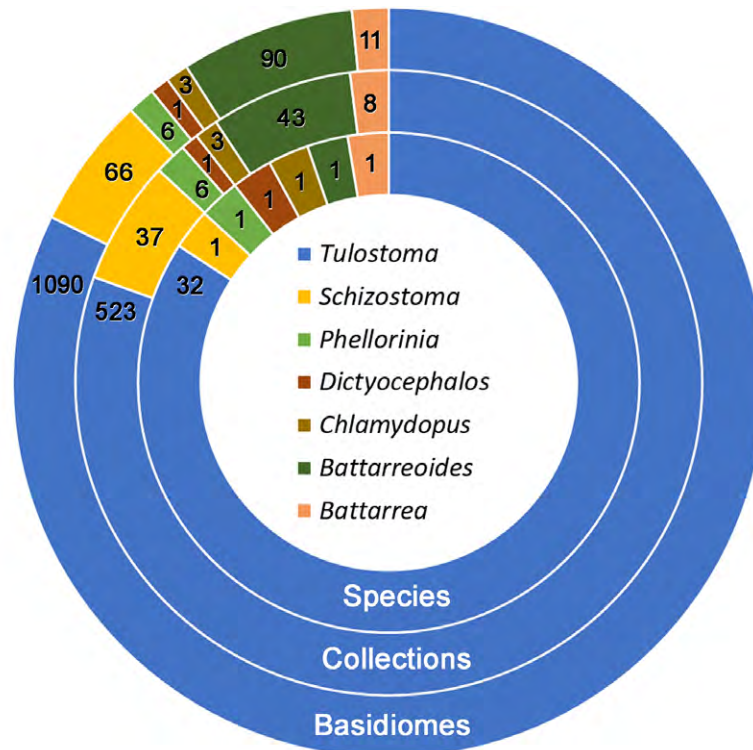


Figure 1. Number of collections, basidiomes, and species per genus.

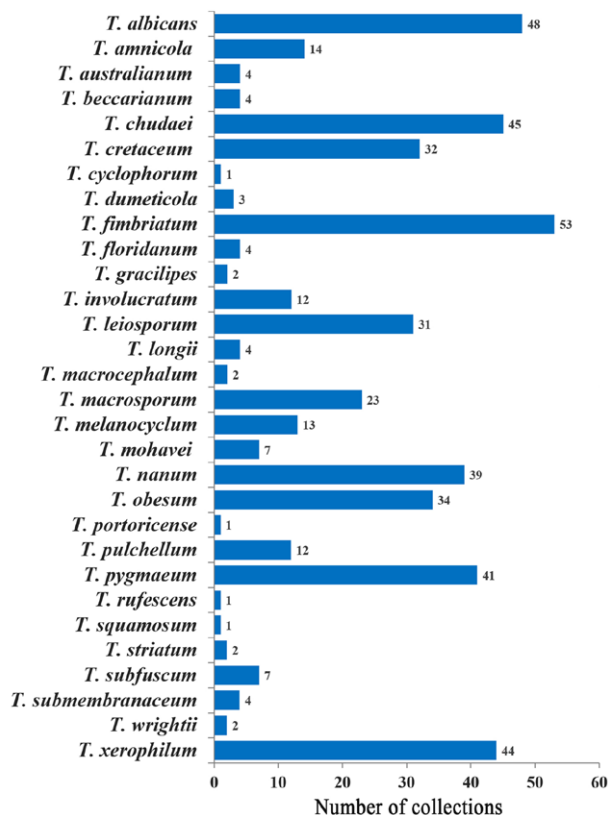


Figure 2. Number of collections of *Tulostoma* species.

The collections were gathered in 75 localities corresponding to 23 municipalities, 16 vegetation types, and 8 soil types with altitudes ranging from 9 to 2,370 m asl. Also, these localities had different pluvial precipitation from 54 to 1,057 mm per year, with 190 mm being the most common. All regimes were associated with precipitation under 700 mm except for locality 75 (1,057 mm). Vegetation type of localities was microphyllous desert scrub (23%), thorn, subtropical, and sarcocaulle scrub (19%), mezquital (16%), sandy desert vegetation (11%), oak forest (7%), tropical thorn forest (7%), natural and induced grassland (2%), pine-oak forest (1%), tropical deciduous forest (1%), halophytic vegetation (1%), and perturbed areas (12%; urban zone, agricultural lands, and cultivated grassland) (Fig. 5). Regions varied from very dry and warm to subhumid temperate. Most collections came from very dry, semi-warm climate. In particular, 65% came from localities considered arid, 27% semiarid, and only 8% from temperate environments.

All TSPs from Sonora are terricolous. Regosol was the most represented soil type with 43% of the localities; 34 out of the 37 identified species were collected in this soil type, with the exception of *D. attenuatus*, *T. macrocephalum*, and *T. portoricense*, which are extremely rare species with only one record each. Yermosol was representative of 17% of the localities and 54% of taxa were collected in this soil type. *Tulostoma melanocyclum* was gathered in

several localities that all had regosol soil type. Meanwhile, vertisol, cambisol, and lithosol represented 17, 15, and 12% of sampled localities, respectively. Notably, *T. cretaceum* was observed in all soil types except lithosol and was the only species found in vertisol. Finally, solonchak and xerosol were present in 4 localities, and vertisol and luvisol were present in one locality.

The first 2 CCA axes had eigenvalues of 0.36 and 0.28, respectively, which accounted together for 60% of total variance in weighted averages of species. Axes were highly significant ($p = 0.001$, 999 permutations in the permANOVA test), which suggests a strong relation between TSPs and environmental conditions on both axes. The first axis had the highest correlation coefficients

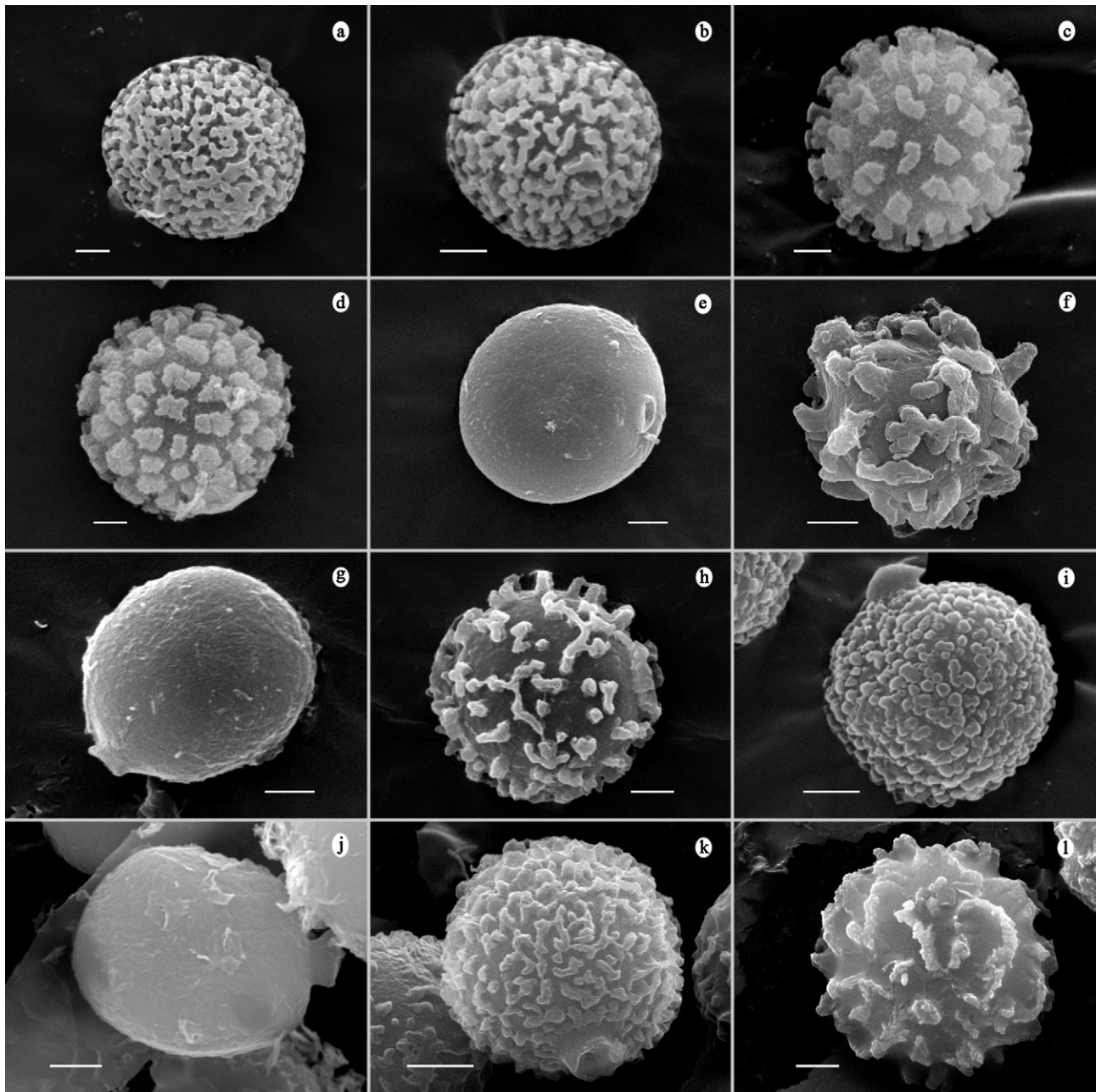


Figure 3. Sporal ornamentation by SEM. a, *Battarrea phalloides* (UES 4861); b, *Battarreoides diguetii* (UES 10479); c, *Chlamydopus meyenianus* (UES 4400); d, *Phellorinia herculeana* (UES 10063); e, *Schizostoma laceratum* (UES 2815); f, *Tulostoma albicans* (UES 10067); g, *T. cretaceum* (UES 10314); h, *T. fimbriatum* (UES 10093); i, *T. gracilipes* (UES 9100); j, *T. leiosporum* (UES 8241); k, *T. longii* (UES 10116); l, *T. macrosporum* (UES 10112). Scale bar = 1 μ m.

with altitude (0.86) and precipitation (0.85), and the second axis with type of climate (0.53). The ordination diagram of CCA shows the main pattern of variation in community composition, which is strongly correlated and best explained by the environmental variables considered, and shows the approximate centers of TSPs and localities distributions along each environmental variable included

(Fig. 6). Many of the TSPs found at the center are ubiquitous species.

The resulting hierarchy tree with TSPs associated with environmental conditions showed 2 clades each with 2 subclades (ScA-ScD), and each subclade with 1, 2, 2, and 3 groups, respectively (GI-GVIII) (Fig. 7). ScA is composed of species with strictly fibrillose to fimbriate

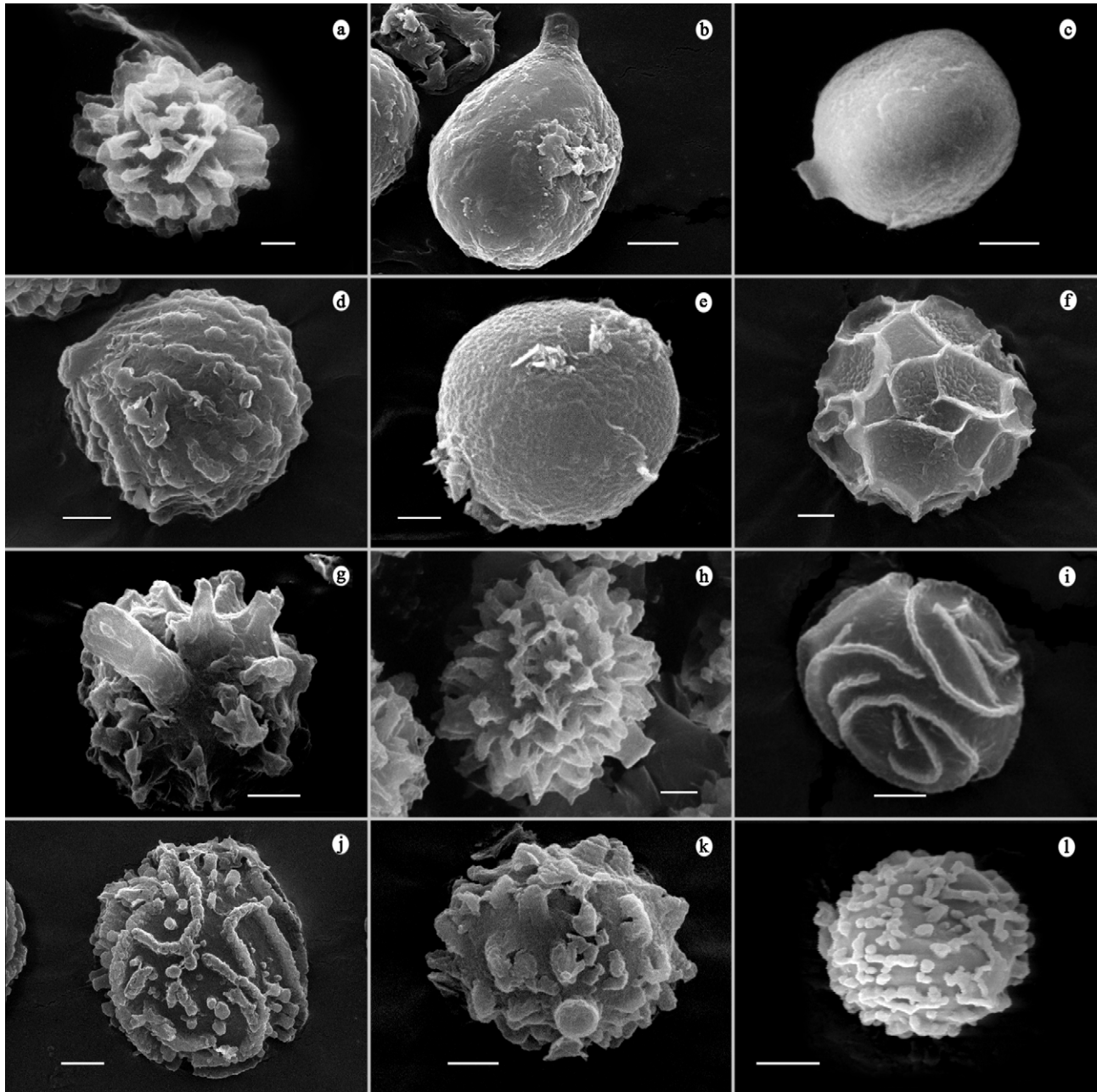


Figure 4. Sporal ornamentation by SEM. a, *T. melanocyclum* (UES 1688); b, *T. membranaceum* (UES 10055); c, *T. mohavei* (UES 5209); d, *T. nanum* (UES 10048); e, *T. obesum* (UES 8321); f, *T. portoricense* (UES 1251); g, *T. rufescens* (UES 10528); h, *T. squamosum* (UES 10092); i, *T. striatum* (UES 5490); j, *T. subfuscum* (UES 10080); k, *T. wrightii* (UES 5608); l, *T. fimbriatum* (UES 906). Scale bar = 1 μ m.

stoma and membranous exoperidia. ScA-GI is composed of species with asperulate to verrucose spores except *T. membranaceum* with smooth spores. ScA-GI also includes rarely collected species with subreticulate, striate, substriated and verrucose spores. ScB-GII is composed of species with asperulate and verrucose spores except

T. portoricense with reticulate spores. ScB-GIII included species strictly with tubular stoma and echinulate spores. ScC included species with smooth spores and indefinite stoma. ScD included species with asperulate to verrucose spores, mainly tubular some fimbriate stoma, and membranous or hyphal exoperidium.

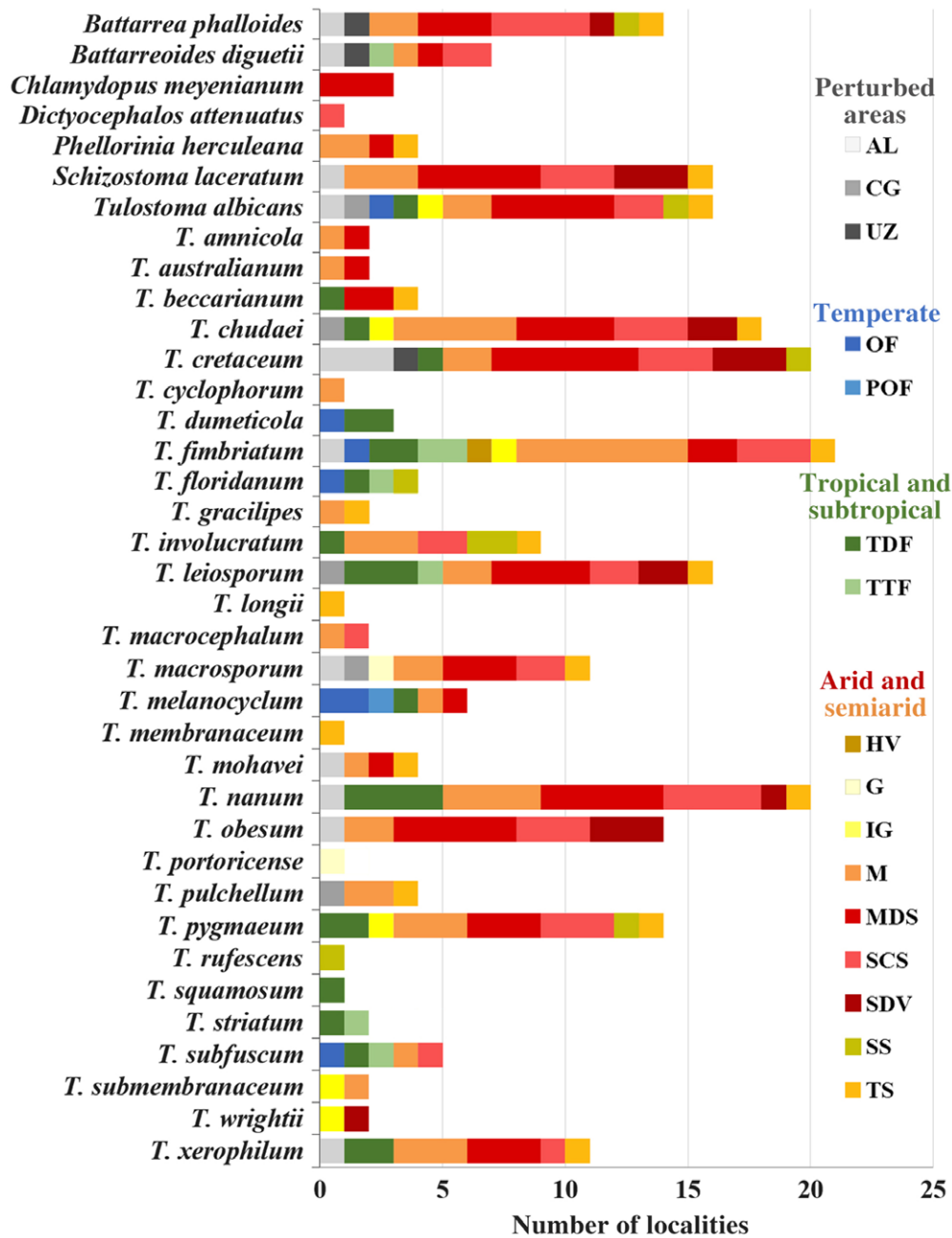
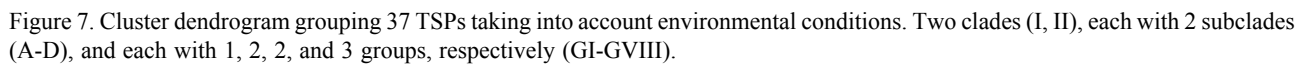
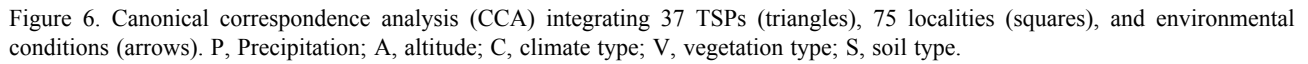


Figure 5. Distribution of tulostomataceous fungi by vegetation type in Sonora. (AL) Agricultural lands; (CG) cultivated grassland; (UZ) urban zone; (OF) oak forest; (POF) pine oak forest; (TDF) tropical deciduous forest; (TTF) tropical thorn forest; (HV) halophytic vegetation; (G) grassland; (IG) induced grasslands; (M) mezquital; (MDS) microphyllous desert scrub; (SCS) sarcocaulle scrub; (SDV) sandy desert vegetation; (SS) subtropical scrub; (TS) thorn scrub.



Discussion

Within the tribe Battarreae, *Battarrea phalloides* and *Battarreoides diguetii* are highly morphologically related. Both species present verrucose spores and elaters in the gleba but are easily distinguished by the pores present in the peridium of *B. diguetii*, while in *B. phalloides*, the dehiscence is characterized by irregular cracks. The exoperidium can be absent in both taxa when mature and intemperized, being difficult to discern. However, *B. diguetii* usually has slender basidiomes with a dry delicate thin volva, whereas the sporocarps of *B. phalloides* can reach up to 65 cm in height and present a conspicuous thick volva that can be gelatinous when fresh (Esqueda et al., 2002). *B. phalloides* has been observed in urban areas of Hermosillo (Esqueda et al., 1995) and recently in both the downtown and suburban area of same municipality. It has also been cited in the urban areas of several other federal entities in Mexico. Even though *B. phalloides* is a cosmopolitan species that has been reported on all continents except Antarctica, it is considered rare and endangered (Rimóczi et al., 2011).

In Mexico, *Battarrea* has been collected between sea level and 2,550 m asl (Esqueda et al., 2002), yet this genus seems to be restricted to arid zones worldwide. However, *Battarreoides* has a more limited distribution. It was first discovered in San Luis Potosí, Mexico, but has since been reported in a few other arid zones of Mexico and Africa (Coetzee & Eicker, 1994; Esqueda et al., 2000; Guzmán & Herrera, 1969). Jacobson et al. (1999) studied the autecology of *Battarrea* and remarked that it is commonly associated with riparian forests on silt floodplain terraces but discarded the existence of mycorrhizal associations with dominant woody species such as *Faidherbia albida* (Delile) A. Chev. or *Tamarix usneoides* E. Mey. Furthermore, there is much discussion about the number of species in *Battarrea*. Currently, more than 15 species have been described. Yet, most mycologists disagree as to whether *B. stevenii* and *B. phalloides* should be considered as 2 distinct species or as monotypic (*B. phalloides*). This has also been argued from a morphological and molecular perspective (Garrido-Benavent, 2015; Martin & Johannesson, 2000; Martin et al., 2013).

The members of Phellorinae share the presence of scales in the peridium that usually fall off, warty and yellowish spores, and persistent, fasciculate basidia at maturity. However, they differ in 3 main aspects: 1) the continuity of the stipe (continuous in *Phellorinia* and discontinuous in the others), 2) the gleba (cellular in *Dictyocephalos* and dusty in the rest), and 3) the mode of dehiscence (occurs in *Chlamydopus* through an apical orifice but is irregular in the others) (Gube, 2009; Long &

Plunkett, 1940; Long & Stouffer, 1946). In *Chlamydopus*, only 2 species are currently accepted, *C. meyenianus* and *C. clavatus*, while *Dictyocephalos* is considered monotypic with *D. attenuatus*. In *Phellorinia*, the variable shape of the scales, among other characters, led to the naming of 21 taxa (15 spp., 2 subspecies, and 4 varieties), although this genus is currently considered monotypic (*P. herculeana*), as discussed by Sharma et al. (2015). Sonoran specimens present peridia with either overlapped or pyramidal scales. The 3 genera have been reported in various countries, but with considerably less frequency than *Tulostoma*.

The 3 genera of Tulostomatinae develop basidiomes with detachable stalks through a socket of spore-sacs that produce abundant capillitia. This is the most diverse group within the TSPs. The main differences between these genera are found in the dehiscence as well as in several microscopic features, such as the type of capillitium and spore ornamentation.

Schizostoma species are characterized by substelliform dehiscence and dark, pulverulent gleba composed of short, dark, and disarticulated capillitial threads at the unswollen septa (*Schizostoma*-type) concolored with the smooth spores as well as a conspicuous volvoid structure that resembles a rhizomorph. There are 2 commonly accepted species worldwide: *S. laceratum* and *S. mundkurii* (S. Ahmad) Long & Stouffer. The latter has also been classified within *Queletia*. *S. laceratum* grows in Sonora, and is morphologically close to some species of *Tulostoma* that inhabit the most arid zones such as *T. cretaceum* or *T. obesum*, which share *Schizostoma*-type characters; mouth trends to indefinite, dark gleba with short pigmented capillitia and smooth, concolor spores. These *Tulostoma* species are difficult to delimit and can usually coexist.

Tulostoma obesum differs in its initially tubular mouth with a thinly membranous exoperidium. Meanwhile, *T. cretaceum* presents a fibrillose mouth and hyphal exoperidium, although it is commonly found weathered with an indefinite mouth, and its exoperidium may be lost or indistinct. *T. cretaceum* also presents a conspicuous rhizomorph that can be lost if carelessly collected. This rhizomorph differs from that of *T. pygmaeum* or *T. rufescens*, which are conformed by thick, loose hyphal filaments strongly intermixed with debris. *T. cretaceum* and *T. albocretaceum* Long & S. Ahmad (the latter is not recorded in Sonora) have rhizomorphs that more closely resemble a main root with attached sand and less organic matter. These 3 species also differ in their mouth, which is fibrillose in *T. cretaceum* and circular with an entire lip in *T. albocretaceum* and *T. obesum*, even though it tends toward indefinite in the 3 species. During its immature stage, *S. laceratum* can be confused with any of the aforementioned taxa but, in its mature

stage, the dehiscence is substelliform with larger spores (Esqueda et al., 2004; Hernández-Navarro et al., 2018; Wright, 1987).

Most *Tulostoma* species have ferruginous to dark brown gleba composed of hyaline to slightly colored capillitial threads that are sometimes swollen at the septa (*Tulostoma*-type) and a regular apical ostiole that can be circular or oval (either planar or tubular), fibrillose (or denticulated, with or without a margin), or indefinite. Some species can form definite ostioles, although these may become indefinite after weathering; in other species, spores are released from a simple thorn-like aperture. Other smooth-spored *Tulostoma* species can be easily separated from the aforementioned species by their light yellowish spores and hyaline to subhyaline underarticulated capillitium with or without swollen septa. Two examples are *T. amnicola* and *T. mohavei*, which are close and not as common as the taxa with dark spores in the most arid zones. Both present a hyphal exoperidium and circular mouth, although the basidiomes of the first are usually slender (< 10 mm vs. > 10 mm) and have smaller spores (3.6–4.1 vs. 3.9–5.5 μm). Because of their similarities, a more in-depth analysis of these species must be performed. *T. leiosporum* also has delicate basidiomes and apparently smooth spores when viewed under light microscopy (LM) but differs due to its fibrillose mouth and asperulate spores, which are only observable under scanning electron microscopy (SEM). *T. membranaceum* has a distinctly membranous exoperidium and fibrillose mouth with slightly colored capillitium with round septa and dark, irregular spores. This latter species is an ill-defined taxon only known in India and doubtfully in the USA (Wright, 1987), so the Mexican material could be a new record for the American continent or even a new species (Esqueda et al., 2004; Hernández-Navarro et al., 2015).

Asperulate to slightly verrucose or spiny sporal species seem to be dominant in semiarid regions, and the boundaries among these taxa are thin. Of these, *T. albicans* and *T. xerophilum* are 2 common and similar species. Both present a typical membranous exoperidium, white endoperidium, tubular mouth, and yellowish spores that vary from subsmooth, asperulate to verrucose. Their main difference is in size; *T. xerophilum* has slender basidiocarps, the exoperidium falls off in large scales and spores are smaller. Meanwhile, in *T. albicans*, basidiomes and spores are usually larger, and the exoperidium falls off in small patches, with some remaining in endoperidium as scales. In our experience, *T. albicans* presents capillitia with slightly swollen and pigmented septa, whereas *T. xerophilum* presents rounded hyaline septa. Some doubtful specimens of *T. albicans* do not share this trait, and in combination with the spore size and ornamentation variability, could

evidence intraspecific morphological variation or a complex cryptic species. Likewise, *T. longii* has circular stoma, asperulate spores, and membranous exoperidium, but spores < 3.5 μm diam., one of the smallest for the genus. Rarely, the stoma loses its lip, appearing fibrillose. *T. involucreum* also has a membranous exoperidium and circular mouth, but the spores usually have a coarser ornamentation with conic verrucae. In addition, its stipe is usually longer. The character that gives this latter species its name is the involute nature of its membranous exoperidium that rolls although some collections lack this character because of weathering. Likewise, this species can be found at higher elevations in less arid regions and is considerably less abundant than the previous 2 species (Esqueda et al., 2004; Hernández-Navarro et al., 2015; Wright, 1987).

Tulostoma submembranaceum presents small basidiocarps but differs from the aforementioned species because it presents slightly verrucose spores and a thinly membranous exoperidium, although it has a fibrillose mouth. It can also be confused with *T. fimbriatum*, which also presents a fibrillose mouth and verrucose spores but differs in its hyphal exoperidium. Although *T. fimbriatum* is considered a cosmopolitan species, it was recently proven to be polyphyletic (Jeppson et al., 2017). Thus, the molecular characterization of this common species and other common species of *Tulostoma* is likely to result in several new records and species in Sonora.

Four similar species that share a membranous exoperidium, fibrillose to denticulate stoma, and verrucose spores are *T. pulchellum*, *T. gracilipes*, *T. subfuscum*, and *T. wrightii*. *T. subfuscum* has been considered a valid species, a variety of *T. pulchellum*, and recently synonymous with *T. punctatum* by Jeppson et al. (2017). According to Wright (1987), *T. subfuscum* presents spores with fused verrucae that are discernible under both LM and SEM, whereas *T. pulchellum* presents independent verrucae as observed in Sonoran collections. Some specimens of *T. subfuscum* could be mistaken with *T. striatum*. In the latter, the ornamentation is ribbed, whereas the former has fused verrucae. The last 3 species have denticulate stoma with delimited peristome. *T. gracilipes* has been cited 3 times worldwide and somewhat matches the description of *T. pulchellum*; however, it has smaller basidiomes, delicate stalk, fibrillose stoma without delimited peristome, rounded unswollen capillitia septa, and slightly ovoid spores. Since the holotype of this species is from South Africa, we cannot discard the possibility that the Sonoran material is a different species or even a small form of *T. pulchellum*. On the other hand, *T. wrightii* presents non delimited denticulate stoma, submembranous exoperidium, and conic wart spores.

Tulostoma nanum s.l. is characterized by its small basidiomes, asperulate spores, tubular mouth, and hyphal exoperidium. Wright (1987) mentioned that the spore ornamentation of the holotype is similar to *T. giovanellae* Bres. (not known in Sonora) and variable in size (up to 8 μ m in diameter). Some Sonoran collections show this character, although others have asperulate spores without a pattern of verrucae, presenting instead an irregular pattern. Therefore, given the heterogeneity of these Sonoran collections, *T. nanum* s.l. is likely to be a species complex. Two other similar common species with circular mouth and hyphal exoperidium from the semiarid zones are *T. chudaei* and *T. pygmaeum*. *T. pygmaeum* and *T. nanum* both present small spore-sacs, but *T. pygmaeum* clearly differs from the rest because its spores have a coarser ornamentation consisting in conic and blunt irregular verrucae that are easily differentiable under LM. In addition, the Sonoran specimens present a conspicuous rhizomorph strongly intermixed with sand and debris, which *T. nanum* lacks. *T. chudaei* is easily distinguished by its acorn-like hyphal exoperidium, conformed of sand particles, and asperulate spores, although its ornamentation seems to vary from asperulate to conic verrucose between collections. Another characteristic that identifies this species is its easily detachable stem; in fact, of 95 specimens, only 25 presented complete stipes, which were sometimes detached. Other species with asperulate spores but stouter basidiomes are *T. macrocephalum* and *T. australianum*. Yet, these differ in their exoperidium (hyphal vs. membranous), mouth (circular vs. fibrillose), and spore ornamentation (appressed verrucae vs. truly asperulate) (Esqueda et al., 2004; Hernández-Navarro et al., 2017; Wright, 1987). A conflictive taxon is *T. beccarianum*, which is characterized by small to medium spore-sacs and a circular mouth, a hyphal to indistinct exoperidium, and verrucose spores. It was synonymized with *T. simulans*, but the latter is considered as a separate species based on molecular data (Altés & Moreno, 1993; Jeppson et al., 2017).

Tulostoma macrosporum is easily recognized by the largest spore size in Sonoran *Tulostoma* species, reaching up to 14 μ m in diameter, although ornamentation can vary from spiny to apparently subreticulate in LM. This species can be found in semiarid areas; meanwhile, other species with coarser ornamentation are more likely to be found in subtropical to temperate areas. Notably, *T. portoricense* was the only species from Sonora with truly reticulated spores. It is found in grasslands in semidry, temperate climates. Meanwhile, *T. squamosum* was collected in 2 localities in tropical thorn forest and tropical deciduous forest, and *T. floridanum*, *T. melanocyclum*, and *T. rufescens* in subtropical or temperate localities.

Several macroscopic traits easily separate *T. squamosum*, including its scaly, reddish brown stipe, and verrucose exoperidium composed of dark, colored, thick-walled cells as mycosclereids. *T. cyclophorum* and *T. dumeticola* share the latter characteristic, but the first species has a fibrillose mouth, uncolored endoperidium, and verrucose, subreticulated spores, whereas *T. dumeticola* presents a chocolate brown exoperidium, slender sporocarps, and spiny, subreticulated spores. Meanwhile, *T. floridanum* presents a grayish exoperidium with a reddish stipe and echinulated spores.

Tulostoma melanocyclum has a characteristically obscure peristome but an uncolored endoperidium. In Sonora, only *T. dumeticola* and *T. rufescens* present a colored endoperidium that is chocolate and pinkish, respectively, while the others have a whitish, grayish, straw to light yellow color. Most collections of these coarser spore ornamented species show a common trait: the gleba portions tend to be cottony and rich in capillitial threads, which are difficult to disarticulate, with smaller numbers of spores. Species from the most arid zones present a dusty gleba, and the capillitial threads are less abundant and totally disarticulated. In contrast, species from semiarid zones present a powdery but spongy gleba with numerous spores and fewer unarticulated capillitium threads. These characteristics could be correlated with the environments in which these fungi are distributed and with the mechanisms of spore dispersion, which are discussed below.

Accordingly, the morphological characteristics of TSPs are very variable, especially in *Tulostoma*. Based on the combination of characteristics (CC) concept, Wright (1987) hierarchized morphological traits in *Tulostoma* as primary (mouth, type of exoperidium, color of the endoperidium, spore size, and ornamentation) and secondary (socket, stipe, capillitium, septa, and lumen). Under this concept, specimens that do not match in any of their primary characteristics are considered separate taxa. Some other characteristics such as size of spore-sac were pointed to as critical, with a slender or robust sac as < 10 or > 10 mm in diameter, respectively. However, some Sonoran collections did show high variability in these characters. For this reason, some “intermediate” specimens, which may likely form part of species complexes, remain unidentified. Despite the great collection efforts made over 29 years, there is still vast territory in Sonora to be explored for new collections.

Certain CC seem to be correlated with the environment and spore dispersion (Gube & Dörfelt, 2012). Wright (1987) described the habitat of *Tulostoma* species according to vegetation and soil type. These species can be classified as psammophilous (present in sandy soils in

arid regions), terricolous (clay-loving species present in pastures or at roadsides), or “forest-soil-loving species” (present in tropical or temperate zones with a high content of organic matter). Wright (1987) also mentioned that *Tulostoma* species are very sensitive to disturbances or modifications to their habitats, such as the loss of plant species, which can result in the absence of basidiomes for 5 to 7 years. However, several *Tulostoma* species were also observed in urban and agricultural zones in Sonora (Fig. 5). Contrary to Wright (1987), the first axis of the CCA had the lowest correlation coefficients with soil (-0.12) and vegetation type (-0.16), while the second axis had the lowest correlation coefficients with vegetation (-0.01) and soil type (0.08) (Fig. 6).

In very dry areas (< 300 mm annual precipitation), spore dispersion is more likely to be air-mediated. In the case of TSPs, almost all genera seem to be euanemochorous, meaning that they present an irregular dehiscence and that the sporocarp is well anchored into the ground. This mechanism is the most common in all genera except for *Battarreoides* and some species of *Tulostoma* with defined ostioles. An irregular dehiscence or indefinite mouth leaves the gasterothecia exposed, and the short, disarticulated capillitia are interspersed among a pulverulent mass of smooth spores with little friction. In our study, subclade C had the lowest number of species (5), all of them with smooth spores; ScC-GIV included *T. cretaceum* and *T. obesum*, which inhabit in the most arid sampled zones with dark yellowish spores and stoma tend to indefinite (Fig. 7). Dark, thick walls of many species may serve to protect from UV damage, as seen in other GSAs from the Sonoran Desert, such as *Calvatia pygmaea* (R.E. Fr.) Kreisel, G. Moreno, C. Ochoa & Altés, *Agaricus deserticola* G. Moreno, Esqueda & Lizárraga, and *Montagnea arenaria* (DC.) Zeller.

In semiarid regions (≥ 300 mm annual precipitation), the boleohydrochorous species present defined mouths and an unarticulated capillitium. This may be one way to protect the gasterothecia and regulate spore release through water droplets. The asperulate to verrucose ornamentation adds friction to the spore mass, so a major force is needed to release the spores. Subclade D included the highest number of taxa (12) and the most common collected species such as *T. albicans*, *T. fimbriatum*, *T. nanum*, and *T. xerophilum*, which have asperulate to verrucose spores and tubular stoma except *T. fimbriatum* and *T. submembranaceum* (fibrillose) (Fig. 7). The required force is greater when capillitia are more abundant

and spores are more ornamented. Coarser ornamentation types might be useful for specific dispersal modes, such as aerial or animal-mediated, as spiny spores are more likely to attach to surfaces. Or, reticulated spores might aid in aerial dispersion. Subclade B-GIII comprised species strictly with tubular stoma and echinulate spores (*T. dumeticola*, *T. floridanum*, *T. melanocyclus*, *T. rufescens*, and *T. squamosum*) (Fig. 7) which inhabit temperate, sub-tropical, and tropical sites (Fig. 5). These dispersal modes are a common feature of species that develop in highlands. However, many tropical and temperate areas in Sonora are located in mountain ranges where slopes are also crucial for spore establishment.

Another strategy is evidenced by the geuanemochorous species with easily detachable stems (e.g., *T. chudaei*) (ScD-GVI; Fig. 7), which allows the spore-sac to tumble freely like some other GSAs common to the Sonoran Desert (e.g., *Disciseda* spp., *C. pygmaea*). The dried spore-sac safely guards the spores and randomly disperses them following interactions with animals or as a result of other factors, such as air and water. The presence of strongly mixed debris in the exoperidium might help the spore sac to gain weight and ensure that the mouth remains facing upwards as in *Disciseda* species (Calonge, 1998; Gube, 2009).

The most abundant species in thorny scrub (e.g., *T. nanum*, *T. xerophilum*; Fig. 5) were commonly found in uncovered areas in poor soils, while other less abundant species were found under the canopy in soils with higher organic matter content. This suggests that the abundant species are well adapted to semiarid environments, which are considered the origin of GSAs (Gube & Dörfelt, 2012). The differences in the abundance and spore dispersal of the studied taxa might also be explained by a combination of environmental conditions and other ecological interactions, such as competition with other decomposers. However, in this regard, the sampling effort is also relevant. For example, locality 47 with thorny scrub was intensely sampled by Esqueda et al. (2000) and Hernández-Navarro et al. (2015), who reported 19 and ~ 50 TSPs and GSAs, respectively, compared to the number of species (10 to 12 GSA species) reported in other nearby localities.

Finally, further molecular analysis of potential new species and basidiomes with intermediate morphology from tulostomataceous stalked-puffballs must be performed in order to determine the real number of species and to understand the boundaries among closely related genera and species.

Key to Sonoran tulostomataceous stalked puffballs.

- 1a. Gleba with elaters 2
- 1b. Gleba without elaters. 3
- 2a. Peridium dehiscence circumscissile, basidiomes stout. *Battarreia phalloides*
- 2b. Peridium dehiscence by multiple definite pores, basidiomes slender *Battarreoides diguetii*
- 3a. Spore-sac not detachable from stalk, peridium covered with pyramidal or overlapped scales..... 4
- 3b. Spore-sac detachable from stalk through a socket, peridium without scales. 6
- 4a. Peridium continuous with stalk, spores verrucose of 4.5-6 μm diam *Phellorinia herculeana*
- 4b. Peridium not continuous with stalk. 5
- 5a. Dehiscence by an apical fibrillose stoma, gleba pulverulent *Chlamydopus meyenianus*
- 5b. Dehiscence by irregular cracks in the peridium, gleba cellular *Dictyocephalos attenuatus*
- 6a. Gleba pulverulent, capillitium disarticulated with some ribbon-like threads. 7
- 6b. Gleba pulverulent to cottony, capillitium non-disarticulated without ribbon-like threads 9
- 7a. Dehiscence by irregular cracks to substelliform at maturity *Schizostoma laceratum*
- 7b. Dehiscence by an apical stoma which becomes indefinite with intemperization 8
- 8a. Stoma circular, exoperidium thinly membranous, without rhizomorph. *Tulostoma obesum*
- 8b. Stoma fibrillose, exoperidium hyphal, stalk with a distinct rhizomorph. *T. cretaceum*
- 9a. Spores perfectly smooth under LM. 10
- 9b. Spores ornamented 13
- 10a. Stoma circular to tubular. 11
- 10b. Stoma fibrillose 12
- 11a. Exoperidium hyphal like an acorn-cup, spore-sac < 10 mm diam., spores 3.6-4.1 μm diam., yellowish *T. amnicola*
- 11b. Exoperidium hyphal non-acorn-cup like, spore-sac > 10 mm diam., spores 3.9-5.5 μm diam., dark yellowish *T. mohavei*
- 12a. Exoperidium membranous, stoma fibrillose, spores smooth under SEM *T. membranaceum*
- 12b. Exoperidium hyphal, stoma fimbriate, spores rugose under SEM. *T. leiosporum*
- 13a. Spores reticulate or subreticulate 14
- 13b. Spores asperulate, verrucose, echinulate, or striate 15
- 14a. Exoperidium hyphal, endoperidium without mycosclereids, spores 6.0-8.0 μm diam., strongly reticulate with long spines with a membrane as wing-like structure *T. portoricense*
- 14b. Exoperidium membranous, endoperidium with mycosclereids, spores 3.5-4.5 μm diam., verrucose-subreticulate. . *T. cyclophorum*
- 15a. Spores striate or nearly, without spines 16
- 15b. Spores asperulate, verrucose or echinulate 17
- 16a. Spores ornamented by continue ribs sometimes forming spirals. *T. striatum*
- 16b. Spores ornamented by fused verrucae simulating ribs and spirals *T. subfuscum*
- 17a. Stoma circular or elliptic, planar or tubular 18
- 17b. Stoma fibrillose or denticulate, with or without a margin 33
- 18a. Exoperidium hyphal or indistinct 19
- 18b. Exoperidium membranous. 24
- 19a. Basidiome stout, spore-sac < 22 mm diam., spores 4.3-6 μm diam., asperulate at LM and verrucae uneven, appressed some anastomosed under SEM *T. macrocephalum*
- 19b. Basidiome slender 20
- 20a. Spores 6-14 μm diam., asperulate to echinulate some subreticulate at LM *T. macrosporum*
- 20b. Spores size average < 6 μm diam. 21
- 21a. Spore-sac usually > 10 mm diam. 22
- 21b. Spore-sac usually < 10 mm diam. 23
- 22a. Exoperidium hyphal like acorn-cup; stalk easily detached from spore-sac, spores asperulate to minutely spiny ... *T. chudaei*
- 22b. Exoperidium indistinct not acorn-cup like, stipe not easily detached, spores with either blunt, digitiform or conic irregular verrucae. *T. beccarianum*

23a. Spores 4.0-5.5 (-7.0) µm diam., asperulate at LM and irregular verrucae arranged in a polar disposition under SEM, stalk without rhizomorph.	<i>T. nanum</i>
23b. Spores 4.5-5.5 µm diam., irregular echinulate, some spiny and some blunt verrucae, stalk with distinct rhizomorph.	<i>T. pygmaeum</i>
24a. Stoma circular, elliptical, planar or tubular	25
24b. Stoma fibrillose, fimbriate, with or without a margin	33
25a. Spores subsmooth, asperulate or verrucose with blunt verrucae	26
25b. Spores with a coarser ornamentation, distinctly spiny to echinulate	29
26a. Exoperidium membranous, papyraceous, falling-off leaving a clean endoperidium	27
26b. Exoperidium membranous, falling-off in patches, remaining in endoperidium like scales	28
27a. Spore-sac usually > 10 mm diam., exoperidium rolling-in the base of endoperidium, stalk > 4 cm long, spores 4.0-5.0 (-6) µm diam.	<i>T. involucreatum</i>
27b. Spore-sac usually < 10 mm diam., exoperidium falling-off remaining in the lower third of white endoperidium, stalk < 4 cm long, spores 3.5-4.7 µm diam.	<i>T. xerophilum</i>
28a. Spore-sac usually > 10 mm diam., spores 4.5-6.5 µm diam.	<i>T. albicans</i>
28b. Spore-sac usually < 10 mm diam., spores 2.8-3.5 µm diam.	<i>T. longii</i>
29a. Peridium with hyphal fascicles resembling mycosclereids of thick wall > 2 µm	30
29b. Peridium without mycosclereids-like structures	31
30a. Exoperidium with protrusions resembling verrucae, spores echinulate to subreticulate	<i>T. dumeticola</i>
30b. Exoperidium verrucose, spores echinulate with spines not coalescing	<i>T. squamosum</i>
31a. Exoperidium hyphal, peristome dark, spores 5.0-8.0 µm diam	<i>T. melanocyclum</i>
31b. Peristome not dark.	32
32a. Exoperidium hyphal, endoperidium grayish, spores with free spines	<i>T. floridanum</i>
32b. Exoperidium thinly membranous, endoperidium pinkish, spores with some spines fusing	<i>T. rufescens</i>
33a. Exoperidium hyphal or indistinct, spores verrucose some irregularly fusing	<i>T. fimbriatum</i>
33b. Exoperidium membranous or submembranous.	34
34a. Exoperidium typically membranous, dark outside and white inside crust, falling-off in big scales or small patches, even with hydrated exoperidium	35
34b. Exoperidium submembranous, not entirely solid-white membrane, which might lose integrity when hydrated ..	37
35a. Spore-sac up to 26 mm diam., stoma fibrillose to simple, spores asperulate	<i>T. australianum</i>
35b. Spore-sac < 15 mm diam., exoperidium falling-off leaving a clean endoperidium	36
36a. Spore-sac < 9 mm diam., stoma fibrillose, spores 4.1-4.9 µm diam	<i>T. gracilipes</i>
36b. Spore-sac > 10 mm diam., stoma denticulate, spores 4.5-6 (-8) µm diam	<i>T. pulchellum</i>
37a. Stoma fibrillose, spores 4.0-6.0 µm diam. minutely verrucose	<i>T. submembranaceum</i>
37b. Stoma denticulate, spores 3.5-5 µm diam. with conic verrucae	<i>T. wrightii</i>

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