CONTRIBUTIONS TO THE STUDY OF GASTEROMYCETES OF PUERTO RICO

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Summary

Battarrea stevenii and Tulostoma cf. *meridionale,* two cosmopolitan taxa are reported as new records for Puerto Rico. Both species were collected from the same disturbed subtropical dry forested area. A partial checklist of the taxa of macromycetes collected from Boquerón Commonwealth- and Laguna Cartagena National Wildlife Refuges is also provided.

Introduction

Gasteromycetes are defined by Hawksworth *et al.* (1996) as a class of Basidiomycota, traditionally based on holobasidiomycetes with basidiospores which are not ballistosporic (strongly correlated with orthotropic spore development). They are mainly terrestrial or hypogeous, some lignicolous or coprophilous, some saprobes and some are mycorrhizal. 'True' gasteromycetes are applied to 8 orders encompassing 30 families, 106 genera (+ 147 synonyms) and 787 species for which affinity is either unknown or not sufficiently evident to be disposed otherwise, among them are the Battarraceae and Tulostomataceae, in the Tulostomatales (Hawksworth *et al.*, 1995). Members of these two families grow in xeric habitats with scarce rainfall and soils that often have a high content of mineral nutrients, especially in deserts from around the world. The use of *Tulostoma* by the North American Ramah Navajo to cure leg bone fractures in sheep, by application either as a poultice or as an infusion, was reported by Burk (1983), as well as the use by Paiute of *Battarrea* and other puffballs for swellings and sores. In Namibia, the spores of a species of *Battarrea* are mixed with oils to form a protective skin ointment and a cosmetic used by the Topnaar people (Pegler *et al.*, 1996). The genera *Battarrea* and *Tulostoma* are poorly known in the Caribbean, where only a few collections of *Tulostoma* and no collections of *Battatrea* have previously been reported. The only *Tulostoma* species previously known from Puerto Rico were *T. berkeleyii* Lloyd, *T. exasperatum* Mont. and *T. volvulatum* Borsch. as summarized by Stevenson (1976). *Tulostoma portorricence* J. E. Wright was described as a new species by Wright (1987), and it was originally collected by N. L. and E. G. Britton in February 1924 from Laguna Tortuguero, located in a subtropical moist forest of the Vega Baja municipality, north of Puerto Rico, growing in a white sandy beach.

Mycological surveys of the Boquerón Commonwealth- and the Laguna Cartagena National Wildlife Refuges are incomplete. A partial checklist of macromycetes (including Basidiomycetes and Ascomycetes) collected from both forests is presented in **Table 1.** We consider the inclusion of this checklist important because recent biodiversity inventories in the island do not include these reserves and because it is difficult to obtain fresh collections in such dry habitats. In addition, conservationists and resident biologists are interested in including a fungal checklist when developing a revised management plan for both forests. Most of the terrain near the wildlife reserves is being subjected to heavy urbanization which will impact the areas before a more complete evaluation and inventory of biodiversity can be carried out.

Materials and methods

Study sites

The Boquerón Commonwealth Wildlife Refuge (18°01' N, 67°09' W) and Laguna Cartagena National Wildlife Refuge (18°01' N, 67°06' W) are part of the subtropical dry forest zone in Puerto Rico **(Fig. 1),** according to the Holdrige system (a classification system for lifezones that is based on potential evapotranspiration, annual rainfall, and temperature) and ecological studies by Ewe1 & Whitmore (1973). Mean annual temperature is 25°C (= 25.1°C at the University of Puerto Rico's Lajas Agriculture Experimental Station, located nearby), and mean annual precipitation is 1100 mm. Showers occur throughout the year, but heavy rainfalls, comprising over 50% of the annual precipitation, occur from September to November, with a second peak usually in May (Díaz-Soltero, 1990). Vegetation is mostly dominated by an introduced species, *Prosopis juliflora* (Sw.) DC. It is common in coastal plains, hills and alluvial zones, especially at saltpeter bed margins. Other species associated with this forest are *Parkinsonia aculeala* L., *Tamarindus indica* L., *Bucida buceras* L., *Guaiacum officinale* L., *and Acacia* spp. Mangroves include species of *Rhizophora, Avicennia, Laguncularia* and *Conocarpus,* which are common in the former forest. Dominant grasses include species of *Panicum, Sporobolus, Andropogon,* and *Paspalum* among others (Toro & Colón, 1986). Cattle exert great influence in the formation of such groves because of their preference for succulents and tender leaves, leaving the thorny species. Through time, the latter species become dominant.

Microscopic methods

The histological technique used to study hyphal arrangement of basidiocarps was described in Nieves-Rivera & Betancourt (1999). Microscopic observations were made using material mounted on slides in 3% KOH, Melzer's reagent or Iactophenol. Color names are given according to Smithe (1976) and designated by a 3-digit number, e.g., (124). Collections were temporarily curated at the Center for Forest Mycology Research, Luquillo, PR (CFMR) before being deposited at the herbarium of the University of Puerto Rico-Rio Piedras (UPRRP).

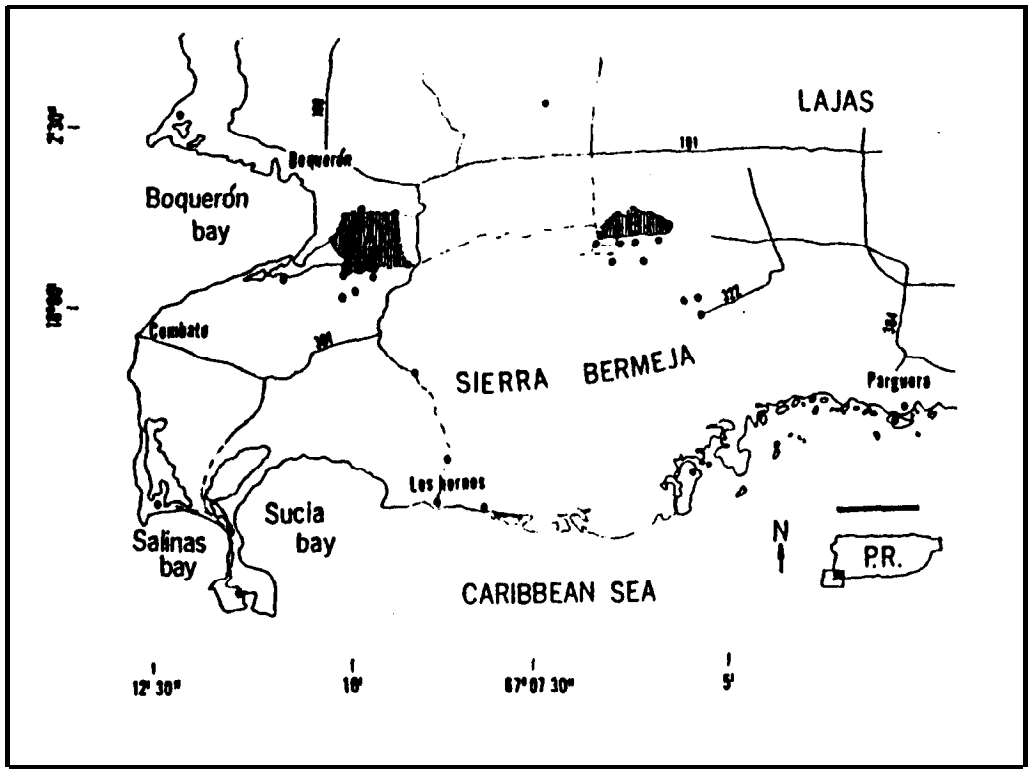

Fig 1. Map of study areas, Boquerón Commonwealth- (A), and Laguna Cartagena National Wildlife (B) Refuges, Cabo Rojo and Lajas municipalities respectively. Collection sites are marked by (·). Shown on the inset is the location of study areas in Puerto Rico. Scale bar = 1 km.

Species collected

Battarrea stevenii (Libosch.) Fr., Syst. Mycol. 3:7 1829.

Basidiocarps 15.5-31.5 cm long including the spore sac, arises from a hypogeous volval cup.

Spore sac 2.0-5.5 cm broad, 2.0-5.0 cm high, subglobose-depressed, splits by circumscissile dehiscence into an upper and lower endoperidium, smooth, then pale true cinnamon, papyraceous to coriaceous, buff when fresh (124), then pale true cinnamon when old. *Upper endoperidium* falls away exposing the gleba. Gleba powdery, rusty brown, burnt sienna (132) when old. *Stipe* 13.5-26.5 cm long, 1.0.-1.5 cm wide, scaly-fibrous, woody, hollow at maturity, tapering somewhat toward base and apex, light buff to pale true cinnamon (139) at maturity, base surrounded by an outer *volva* 4.6-6.2 cm broad, 3.8-4.0 cm high, free, fleshy young, hypogeous, very fragile at maturity composed of an *exoperidium* which is membranous and white and a *mesoperidium* which is thick and fleshy at first but shrinking and brittle-papyraceous in age, often missing with old specimens **(Fig. 2A).**

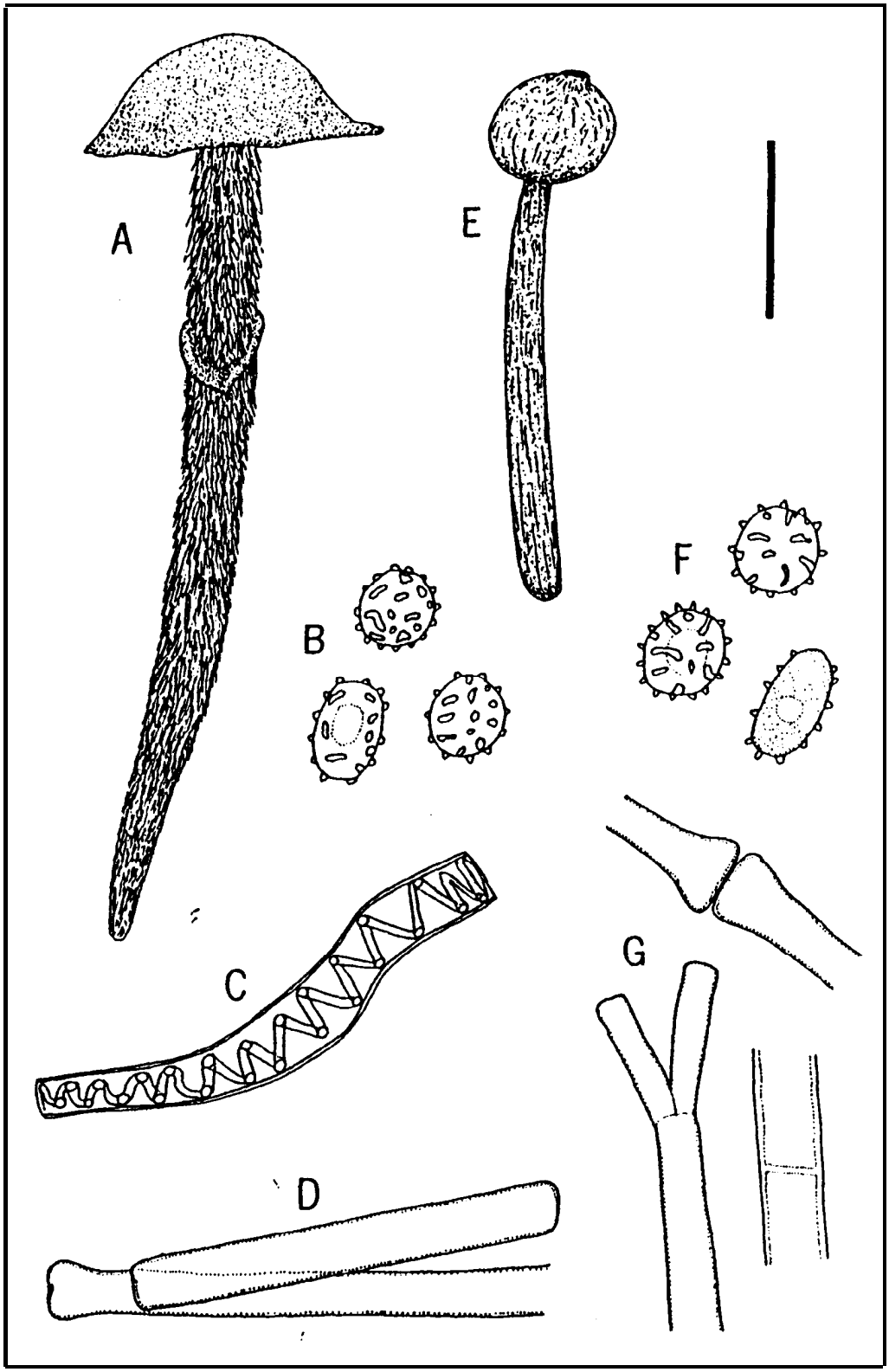

Fig 2. A-D *Battarrea stevenii.* (A) Mature basidiocarp; (B) basidiospores; (C) elaters; (D) pseudocapillitial threads. E-G *Tulostoma* cf. *meridionale.* (E) Mature basidiocarp; (F) basidiospores; (G) Capillitium threads and septa. Scale bar = 68 mm for A; 15 µm for B and F; 18 µm for C; 12 µm for D; 6 mm for E; G not to scale. All illustrations from Nieves-Rivera PR 430 and 431.

Endoperidium consisting of hyphae 3 µm diam, thin-walled intermingled with thick-walled cells, septate, branched, pale yellow, with clamp-connections in 3% KOH. *Gleba* composed of paracapillitium 2.6-5.3 µm diam, smooth, septate, sparsely branched hyaline to pale yellow, equal or slightly broadened at the septa with scattered clamp connections **(Fig. 2D).** Elaters intermixed throughout the gleba 38-77µm long, 5.3-8.0 µm diam wide, cylindric, thick-walled, the entire length with nearly hyaline to pale yellow, spiraled thickenings, in 3% KOH and Melzer's solution **(Fig. 2C).** *Basidia* not observed. *Basidiospores* 5.3-8.0 x 4.0-6.7 µm globose, to subglobose, densely verrucose, thick-walled, pale yellow to yellow-brown in 3% KOH and Melzer's solution **(Fig 2B).**

Odor like codfish, sometimes rotten *or* fetid.

Habitat: usually solitary, appearing after a heavy rainfall, growing in disturbed, reddish soil in an area used for cattle pasture, a sunny open field, a soil bank, and under *Tamarindus indica* association at 6 m alt.

Material examined: PUERTO RICO. Cabo Rojo municipality, Sector El Caño, next to Boquerón Wildlife Refuge (17°59'56"N, 67°09'50"W), 5 July 1994, Nieves-Rivera PR 707 (MAPR); 2 February 1995, Nieves-Rivera PR 709 (CFMR); 12 September 1996, Nieves-Rivera PR 708 (CFMR); 10 January 1997, Nieves-Rivera PR 431 (NY).

Remarks: Basidiocarps have now appeared in the same 5 m^2 area, for three successive years, in an elliptical formation (fairy ring?) under *T. indica.* Jacobson *et al.* (1998) reported *B. stevenii* in ephemeral river beds in Namibia with a high silt fraction exclusively on floodplain terraces and river banks. Soil organic material of 3.6-7 % is found in these habitats. The volva is missing in the illustrated specimen **(Fig. 2A).** Moreno *et al.* (1995) reported *B. stevenii* forming fairy rings. Dittmer (1978) indicated *B. phalloides* (Dicks.: Pers.) Pers. occurred in small numbers and was very sparsely distributed. *Battarrea stevenii* collected from Puerto Rico followed the same pattern as reported by both.

Tulostoma cf. *meridionale* J. E. Wright, in Wright et al., Ciencia (Mexico) 27(4-5): 117 (1972).

T. utahense J. E. Wright, Biblioth. Mycol. 113: 204-206 (1987).

Basidiocarp 25 mm long, at first hypogean. *Spore sac* 5 mm diam, globose, white (dirty white) **(Fig 2E).** Ostiole fibrillose and slightly raised with age. *Exoperidium* membranous, mixed with sandy soil grains, consisting of hyphae 3 µm diam, thin-walled, septate, branched, hyaline, clamps absent. *Endoperidium* smooth, membranous, white to cream, consisting of hyphae 3-6 pm diam, intermingled, thick-walled, septate, broadened at the septa, branched, hyaline, clamps absent. *Gleba* ferrugineous. *Stipe* central, 20 x 2 mm, tapered at base, white (concolorous with spore sac), slightly fibrillose, fibrous, solid, 1/4 inserted in substrate, mycelial cord not seen. *Basidiospores* 6.7-9.3 x 5.3 µm without the warts (warts 0.8-1 µm long), with an average size of 7 x 5.3 µm, globose to ellipsoid or ovoid, verrucose, warts joined sometimes forming short ribs but not reticulated, pale yellow **(Fig 2F).** *Capillitial threads* 5.3-8 µm diam, branched, hyaline, smooth, thin-walled, usually broadened at the septa **(Fig 2G).** *Odor* fetid.

Table 1. Summary of the taxa of macromycetes from field collections in the Boquerón- and Laguna Cartagena National Wildlife Refuges from January to December 1997.[1]

Fungus	Substrate[2]	Collection No. (Nieves-Rivera PR)
BASIDIOMYCETES		
Auricularia delica (Fr.) Henn	W5	755
Battarrea stevenii (Libosch.) Fr.	S	431, 707, 708, 709
Calvatia cyathiformis (Bosc) Morgan	S	744, 765
Ceriporia xylostromatoides (Berk.) Ryvarden	W1, 2	632
Coprinus cf. *plicatilis* (M.A. Curtis: Fr.) Fr.	S	786.2
Cyathus cf. *striatus* Huds.: Pers.[3]	C	766.2
Dacryopinax spathularia (Schw.) G. W. Martin	W5	439, 756
Ganoderma lucidum (Leyss.: Fr.) Karst.	W5	753
Gloeophyllum striatum (Sw.: Fr.) Murrill	W5	435
Hexagonia hydnoides (Fr.: Sw.) M. Fidalgo	W1, 2	631, 641, 748, 760
Leucoagaricus hortensis (Murrill) Pegler	S, C	445
Marasmius pallescens Murrill	C, L	432, 450
Panaeolus antillarum (Fr.) Dennis	C	449
Phellinus dependens (Murrill) Imaz.	W5	633
Phylloporia chrysita (Berk.) Ryvarden	W1	635, 637, 758
Pleurotus djamor (Fr.) Boedijn	W1	447, 747
Pycnoporus sanguineus (L.: Fr.) Murrill	W5	745, 759
Schizophyllum commune Fr.: Fr.	W1, 2, 4	436, 634, 636, 749, 754
Trametes elegans (Spreng.: Fr.) Fr.	W5	444, 629, 763
Tulostoma cf. *meridionale* J. E. Wright	S	430
ASCOMYCETES		
Hypoxylon sect. *Hypoxylon*	W3, 4, 5	644
Poronia oedipus Mont.	C	441, 448

[1]Arbelo *et al.* (1989) found 10 species of mitosporic fungi in soil, next to the eastern side of Laguna Cartagene (*Aspergillus* sp., *Cladosporium* spp., *Fusarium* spp., *Geotrichum* sp., *Helicomyces* sp., *Neurospora* sp., *Penicillium* sp., and *Sporobolomyces* sp.). Eight species of yeasts of aquatic origin were identified, among them *Rhodotorula* sp. (Díaz-Soltero, 1990).

[2]Substrate: C = cow dung: L = leaf litter; S = soil; W = dead wood (W1 = *Ceiba peltandra* L.; W2 = *Prosopis juliflora* (Sw.) DC; W3 = *Rhizophora* mangle L.; W4 = *Avicennia nitida* Jacq.; W5 = undetermined).

[3]Baridiocarps also grew on top of an ant nest (*Solenopsis* sp.), enriched with cow manure.

Habitat: usually solitary, in sunny open field, at 5 m alt. in disturbed, reddish soil with grass in a cattle pasture, after a heavy rainfall.

Material examined: PUERTO RICO, Cabo Rojo municipality, Sector El Caño, next to Boquerón Wildlife Refuge (18°01'00"N, 67°09'53"W), 10 January 1997, Nieves-Rivera PR 430 (CFMR).

Remarks: This collection was originally thought to be *T. berkeleyii sensu* Coker & Couch (1928), but careful examination revealed differences in spore size which is smaller in the former, 3.5-6.0 µm, than in *T. meridionale,* 7-10 (-11.2) µm. Also, *Tulostoma* collected from Puerto Rico have similar morphology and morphometry to *T. meridionale* reported by Wright (1987), Raithelhuber (1991) and Moreno *et al.* (1995). Spore sizes are also different from those *of T. volvulatum* (var. *volvulatum* J. E. Wright, var. *elatum* Hariot & Pat., and var. *obesum* (Cooke & Ellis) J. E. Wright, which are 5-5.5 µm in average). *Tulostoma metidionale* was previously recorded from the southern United States, Mexico and Argentina (Wright, 1987; Moreno *et al.* 1995), growing in humus (Raithelhuber, 1991) and sandy soil (Wright, 1987).

Partial checklist

Basidiomycetes and ascomycetes collected from both forests are summarized in **Table 1.** They are cosmopolitan and grew in four types of substrates: (a) wood, (b) cow dung, (c) soil, and (d) leaf litter. Most collections were on dead and decaying wood, and include species in the Polyporaceae/Coriolaceae (8), Tricholomataceae (3), Coprinaceae (2), Auriculariaceae (1), Dacrymycetaceae (1), Schizophyllaceae (1), Lycoperdaceae (Basidiomycota)(1) and Xylariaceae (Ascomycota) (3). In addition, other collections in both *reserves,* on dead and decaying wood include members of the Polyporaceae/Coriolaceae, Thelephoraceae (Basidiomycota), Xylariaceae, Thyridiaceae, Strigulaceae, Pyrenulaceae, Hysteriaceae, Boliniaceae and Pleurotremataceae (Ascomycota).

Discussion

Species of *Battarrea* have been observed growing under trees in pure silica sand close to river banks, deserts and disturbed land (Jacobson, 1996; Jacobson et al., 1998). Pegler *et al.* (1996) state that *B. phalloides* is sometimes associated with trees such as *Fraxinus, Taxus,* and *Pinus.* This fungus species is also reported from Africa, Australia, Europe, and North America, but records often include *Battarrea stevenii* and are difficult to assess, particularly the North American collections (Pegler *et al.,* 1995; Moreno *et al.,* 1995). Perreau (1986) suggested that *B. phalloides* is a synonym of *B. stevenii.* We agree that *B. phalloides sensu stricto* is most likely the same as *B. stevenii,* but this question must await further studies of the holotypes as suggested by Moreno *et al.* (1995). We considered it plausible that morphological variations between these two species might simply be variation associated with climatic and soil conditions. While studying fungal ecology in the Namib Desert, Jacobson (1996) collected and cultured abundant fresh specimens of *B.* cf. *phalloides* (= *B. stevenii*) during the most recent flooding of the river channels. This species has been reported as far

as Yukon, Canada. Future studies will include molecular taxonomy and phylogeny of *B. stevenii* by Jacobson and Miller. The species, *B. phalloides* is in question since it is described as having a gelatinous volva in the button stage. Buttons examined in Namibia, Idaho and southern Europe showed a firm interwoven non-gelatinous volva. It has a widespread world distribution and without any contrary evidence the taxon in Puerto Rico and the Antilles in general would most likely be *B. stevenii.* Collecting a button is not very likely because it is necessary to mark the exact site where it is found and dig for them about 6-8 weeks prior to the date of collection of mature specimens.

The genus *Tulostoma* has been reported from both Paleo– and Neotropics and temperate regions, but mainly from warm dry regions (Hawksworth *et al,*. 1995); ranging from sea level in sandy areas, but also in interior deserts and arid mountains up to 2743 m alt. *Tulostoma* is an interesting genus in which little is known, Except for their long, fibrous stipes, they usually resemble simple, small puffballs and because of their small size they are difficult to detect. Also, many *Tulostoma* spp. are difficult to identify in the field because distinguishing characteristics are mostly microscopic. Perhaps all these aspects caused the lack of interest and no further collections of *Tulostoma* spp. have been obtained from Puerto Rico since the original report by Coker & Couch (1928).

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