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Description and life cycle of a new *Didymium* (Myxomycetes) from arid areas of Argentina and Chile

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Abstract: A new species of *Didymium* (Myxomycetes), *D. infundibuliforme*, is described herein, and details are provided on its life cycle as observed in spore to spore culture on agar. The new species was recorded during intensive studies of areas of the Monte Desert in Argentina and the Atacama Desert in Chile. It has been collected directly in the field in both countries on several occasions over 4 y and isolated in moist chamber cultures prepared with material from native plant species. The characters that make this species unique in the genus are its funnel-shape sporocarps with white stalks, the apical circumscissile dehiscence of the sporotheca that causes the base to resemble a calyculus and the ornamentation on the spores. The morphology of specimens of this new myxomycete was examined with scanning electron microscopy and light microscopy, and micrographs of relevant details are included in this paper.

Key words: agar culture, deserts, morphogenesis, Mycetozoa, *Puya*, SEM, taxonomy

INTRODUCTION

The myxomycete genus *Didymium* originally was described by Schrader (1797) and now includes approximately 70 species (Lado 2001, 2008, Hernández-Crespo and Lado 2005), about 25% of which have been described in the past 20 y. Myxomycetes in this genus are characterized by sporophores with a peridium covered with stellate crystals of calcium carbonate, with these sometimes forming a crust of aggregated crystals. In contrast the capillitium is almost always limeless. The crystalline nature of the lime on the peridium separates *Didymium* from *Diderma*, in which the lime occurs as amorphous granules (Martin and Alexopoulos 1969). It has become evident that a group of species within the relatively large genus *Didymium* are characteristically associated with plants in arid areas of the world. This paper describes another new *Didymium* that belongs to this ecological group, and details are provided on its life cycle as observed in spore to spore culture on agar. The new species was obtained during intensive studies of arid areas of Argentina and Chile, in Monte and Atacama deserts (Lado et al 2007a). It has been isolated in moist chamber cultures both native and endemic plant species, as well as being found directly in the field in both countries on several occasions over 4 y.

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MATERIALS AND METHODS

Collecting sites were located 18–31°S and 65–70°W, in areas, many of them protected, where the vegetation is relatively little affected by anthropogenic activities. In northwestern Argentina sites were located along the eastern foothills of the Andes (1000–2100 m). The vegetation is predominantly xerophyllous scrubland with rosette-leaf succulent plants such as *Puya* spp. and desert scrubland dominated by cacti such as *Tephrocactus* spp. and *Trichocereus* spp . In central and northern Chile the study areas were at the southern limits of the Atacama Desert, along a large altitudinal gradient, from sea level to 3300 m. The vegetation is dominated by xerophyllous scrublands, which include endemic species of cacti such as the globose *Copiapoa* spp. and candelabra cacti such as *Eulychnia* spp. At higher latitudes were species of the bromeliad genus *Puya*, the dead and decaying leaves of which we have found to be a remarkable substrate for myxomycetes, including the species described here.

The surveys involved collecting myxomycetes in the field from known or suspected microhabitats, along with the removal of substrates for laboratory culture. This paper is based on material obtained from field collections in xerophyllous scrubland and moist chamber cultures prepared with leaf bases of *Puya* spp., which were collected in four states of Argentina (Catamarca, Jujuy, La Rioja and San Juan) and the first three regions in Chile (Antofagasta, Atacama and Tarapacá), in addition to the material obtained from spore to spore cultures on agar. Field collections and substrate material for moist chamber cultures were obtained on four occasions, Jan 2004–Mar 2008. Field collections were glued into herbarium boxes and dried in situ. Material for preparation of moist chamber cultures was air-dried in situ and transported to the laboratory in sealed paper bags. All localities were

geo-referenced with a GPS (Magellan eXplorist 600 5.1, Datum WGS84).

Agar cultures were prepared with isolates (dwb 07110 and dwb 0806) derived from mature sporocarps of collections dwb 2825 and dwb 2834. The spores were sown on 0.75% water agar (WA) at pH 7.0. The sporocarps were crushed and spores released over the agar in each of four quadrants of sterile 9 cm plastic Petri dishes. Agar cultures were kept at room temperature (21–23 C) with an approximate 12 h light-dark regime. Details of the media used and techniques followed can be found in Haskins and Wrigley de Basanta (2008). In addition slide cultures (Spiegel et al 2005) were prepared for some of the light micrographs of germinating spores and amoebae. Moist chamber cultures were prepared with pieces of dry *Puya* spp. leaf bases, which were placed on filter paper lining sterile 9 cm plastic Petri dishes. The samples were soaked with boiled tap water (from Madrid), and excess water was removed after 24 h after the pH of each culture was determined and recorded. Cultures were kept 3 mo at room temperature (21–25 C) in diffuse daylight. The cultures were examined daily with a stereoscope for the first week and every 2–3 d after that for the culture period. Samples were kept moist by adding small amounts of boiled tap water as necessary. The date that mature sporocarps developed was recorded, and they were removed on small pieces of substrate, dried slowly in closed sterile Petri dishes and glued into small boxes. All sporocarps of the same species in one culture were regarded as representing one collection. All specimens are deposited in the herbaria MA-Fungi (sub Lado) or the private collection of the first author (dwb).

All microscope measurements and observations were made with material mounted directly in Hoyer's medium. A microscope with differential interference contrast (DIC) was used to obtain descriptive data and light micrographs. Measurements were made of 10 spores from each of the collections. Critical point drying was used for scanning electron microscopy (SEM) preparations, and the SEM analyses and photomicrographs of specimens were made by the Scanning Electron Microscopy Department of the Royal Botanic Garden of Madrid, employing a Hitachi S-3000N scanning electron microscope at 10–15 kV. Color notations in parentheses are from the ISCC-NBS color name charts illustrated with centroid colors (Anonymous 1976).

RESULTS

Twenty-three collections of this new species have been obtained, nine as field collections and 14 collections from moist chamber cultures. The collections are from states in two countries and were obtained over 4 y. The life cycle of this myxomycete has been observed on agar from spore to spore, and this has demonstrated the stability of its taxonomic characters. As such it is described herein as new to science.

Didymium infundibuliforme D. Wrigley, Lado et Estrada, sp. nov. FIGS. 1–25

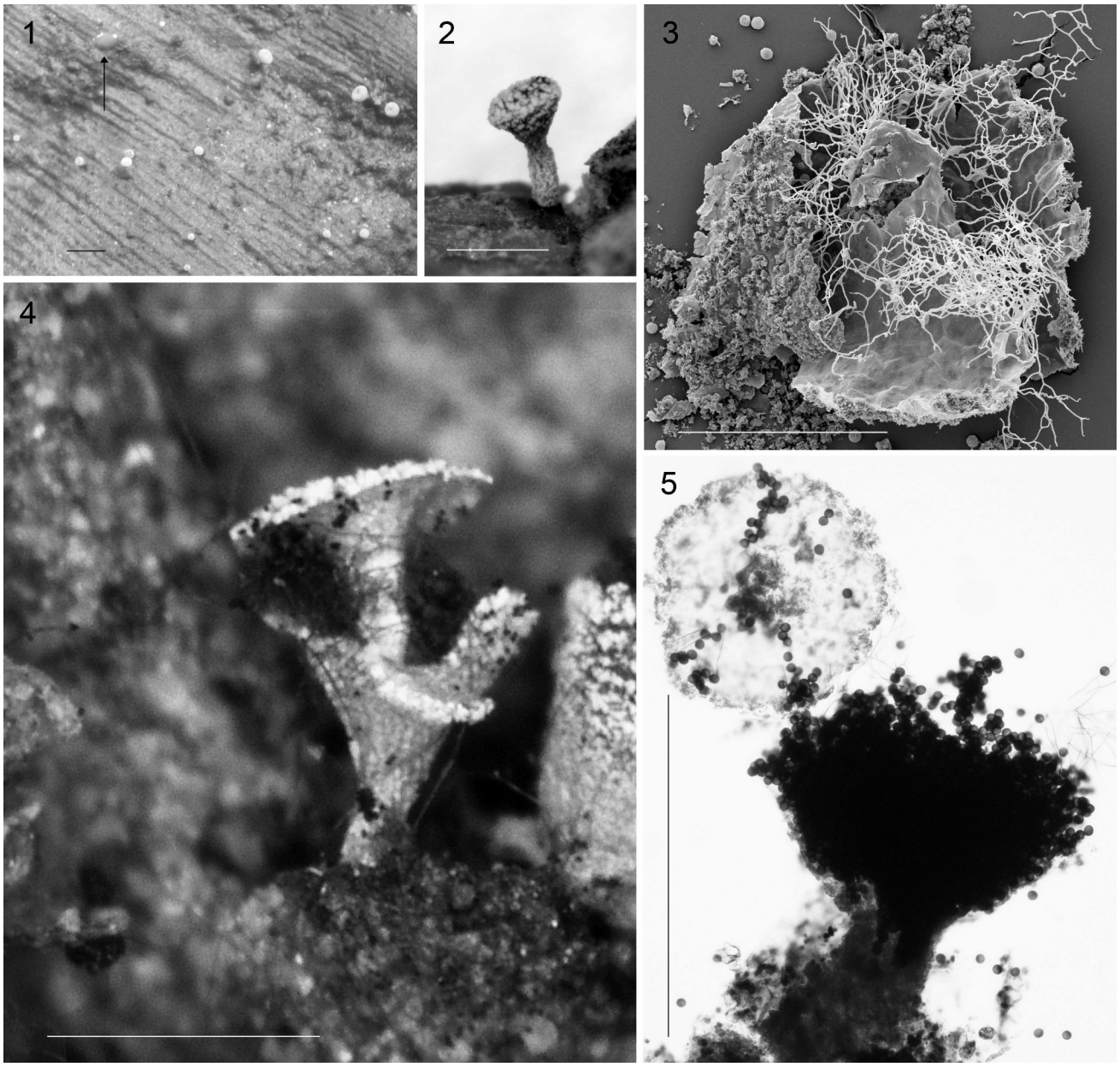
Mycobank MB513118

Sporophora sporocarpica, dispersa vel aggregata. Sporocarpia infundibuliformis, 0.2–0.6 mm alta, breviter stipitata. Sporotheca obconica, 0.15–0.8 mm diam, distaliter invaginata, pallidissime grisea vel prorsus alba. *Stipes* calcareus, erectus, parvus, 0.04–0.3 mm altus, 0.05–0.14 mm latus. *Peridium* unicum, membranaceum, crystallis albis, calcareis, coopertum. Dehiscentia circumscissa. Sine columella. *Capillitium* filamentosum, filamentis 1–2 μm diam, parce ramificatis, erectis vel undulatis. *Sporae* liberae, griseobrunneae, (11–)12–13(–15) μm diam, subglobosae, verrucosae.

Sporophores sporocarpic, dispersed or grouped. Sporocarps funnel-shape (FIGS. 1–2), 0.2–0.6 mm high, with short thick stalks. Sporotheca obconic, 0.15–0.8 mm diam, with the apex invaginated to form a funnel (FIG. 4), covered with calcium carbonate crystals, light gray (264. l. Gray) to white (263. White) especially above, iridescent below when crystals are sparse or absent. Stalk erect, short, 0.04–0.3 mm high, 0.05–0.14 mm wide, calcareous, with lime crystals inside and on the surface, ending in the restricted base of the sporotheca, yellowish white (92. y White) to white (263. White) by LM. Peridium single, membranous, colorless to yellowish white (92. y White) by LM, its inner surface smooth, also by SEM (FIG. 3), covered by white, stellate lime crystals; crystals forming a roughened layer (FIGS. 2, 4), sometimes scattered showing the iridescent peridium; dehiscence circumscissile at the apex of the sporotheca (FIG. 5). True columella absent, but an invagination of the peridium reaches the thickened base of the sporotheca, forming a funnel (FIG. 4). Capillitium filiform (FIGS. 9–10), threads 1–2 μm diam, branched, straight to undulating, with cross connections, grayish reddish brown (46. gy. r Br) to grayish brown (61. gy. Br), smooth (FIG. 10). Spores free, black in mass, grayish reddish brown (46. gy. r Br) to grayish brown (60. l. gy. Br–61. gy. Br) by LM, subglobose, (11–)12–13(–15) μm diam, warted under LM (FIG. 13), with verrucae and bacula interconnected at their bases by an irregularly meshed net of bands by SEM (FIGS. 6–8, 11–12, 25). Plasmodium a small phaneroplasmodium, hyaline to milky.

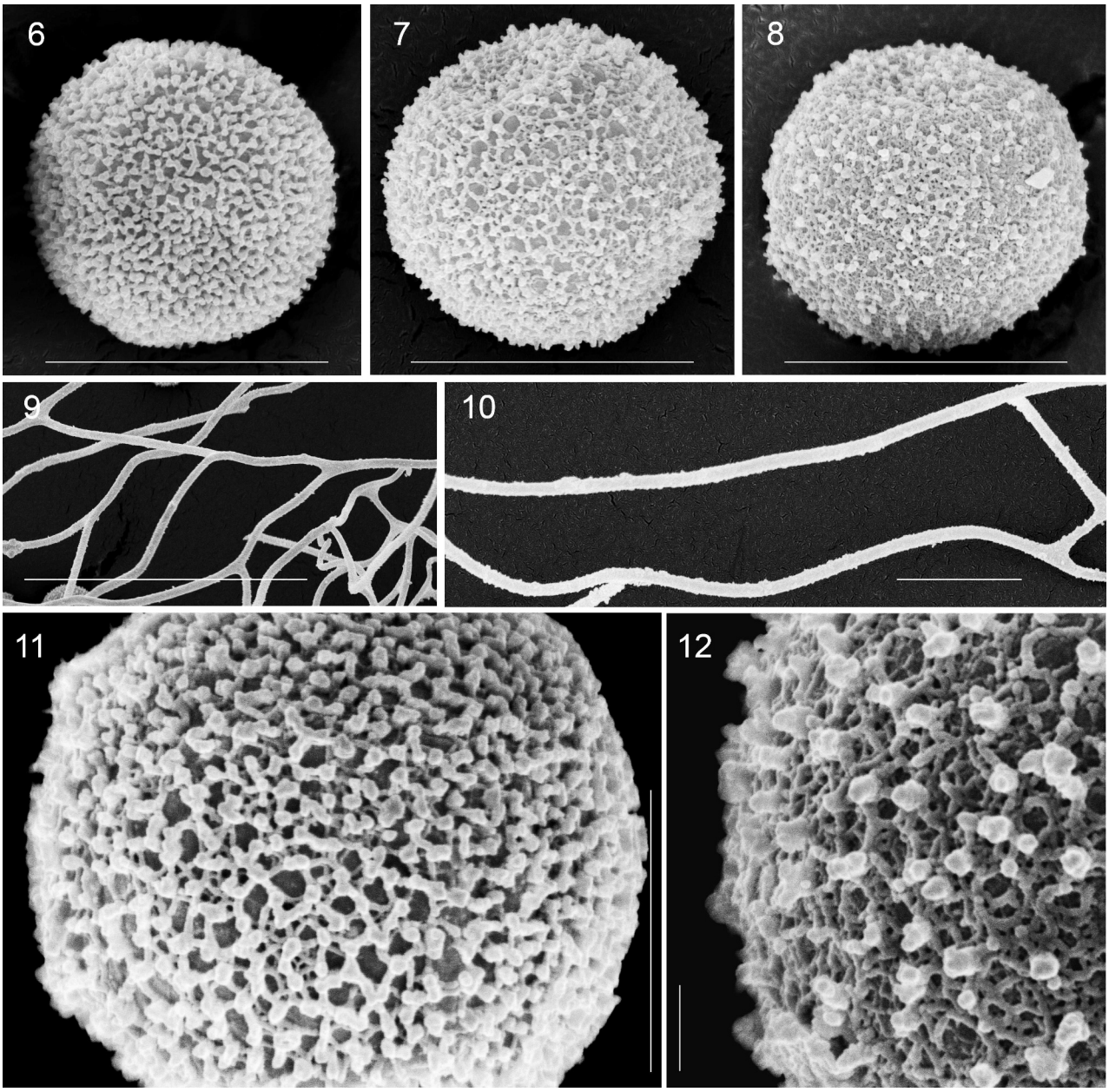
HOLOTYPE. ARGENTINA. Catamarca, Costa de Reyes, Route R-3, 28°16′18″S 67°38′51″W, 1437 m ± 7 m, 29-XI-2006, on dead leaf bases of *Puya* sp., *D. Wrigley de Basanta, C. Lado & A. Estrada-Torres*, Lado18374 (MA-Fungi 78320).

Specimens examined. ARGENTINA. Jujuy: Volcan, Tumbaya, Huajra, 23°52′12″S 65°27′50″W, 2112 m, 7-IX-2007, on dead leaf bases of *Puya* sp. in moist chamber culture (pH 6.9), dwb 2942. Catamarca: Belén, Morro de los Cóndores Nature Reserve, 27°34′13″S 67°00′10″W, 1308 m, 12-III-2007, on dead leaf bases of *Puya* sp. in moist chamber culture (pH 6.8), dwb 2829; ibidem, 13-III-2007, dwb 2834.



FIGS. 1–5. *Didymium infundibuliforme*. 1. Sporocarps and plasmodium (arrow) on *Puya* sp. leaf base in moist chamber culture (dwb 3044). Bar = 1 mm. 2. Sporocarp (dwb 2825). Bar = 0.5 mm. 3. Sporocarp with spores and capillitium as viewed by SEM showing smooth inner surface of peridium (dwb 2554). Bar = 200 µm. 4. A dehisced sporocarp showing the apical circumscissile dehiscence and the funnel-shape invagination of the peridium that penetrates the remaining base of the sporocarp (Lado 18374). Bar = 0.5 mm. 5. Dehisced sporocarp by transmitted light showing circular lid with invaginated peridium and funnel-shape base (dwb 2843). Bar = 0.5 mm.

Belen, Route RN-40 from Belen to Hualfin, 27°36′55″S 67°01′06″W, 1305 m, 27-XI-2006, on dead leaf bases of *Puya* sp., Lado 18313 (MA-Fungi 78321); 7-XII-2007, on dead leaf bases of *Puya* sp. in moist chamber culture (pH 6.8), dwb 3019. Tinogasta, La Puntilla, 28°06′13″S 67°30′52″W, 1184 m, 11-III-2007, on dead leaf bases of *Puya* sp. in moist chamber culture (pH 7.1), dwb 2851. Costa de Reyes, Route R-3, 28°16′18″S 67°38′51″W, 1437 m ± 7 m, 29-XI-2006, on dead leaf bases of *Puya* sp., Lado 18369 (MA-Fungi 78322), Lado 18372 (MA-Fungi 78323), Lado18374 (MA-Fungi 78320). La Rioja: Independencia, Talampaya National Park, 30°07′42″S 67°44′19″W, 1378 m, 11-III-2007, on dead leaf bases of *Puya* sp. in moist chamber culture (pH 6.9), dwb 2825; 18-III-2007, (pH 6.8), dwb 2845; 23-III-2007, (pH 7.2), dwb 2843. San Juan: Jáchal, San Roque, km 3619 of Route RN-40, 30°21′03″S 68°38′79″W, 1054 ± 5 m, 8-III-2007, on dead leaf bases of *Puya* sp., Lado 18708 (MA-Fungi 78324), Lado 18712 (MA-Fungi 78325). Ullum, Termas de Talacasto, 31°01′41″S 68°45′44″W, 1333 m, 2-VI-2007, on dead leaf bases of *Puya* sp. in moist chamber culture (pH 6.4), dwb 2894.

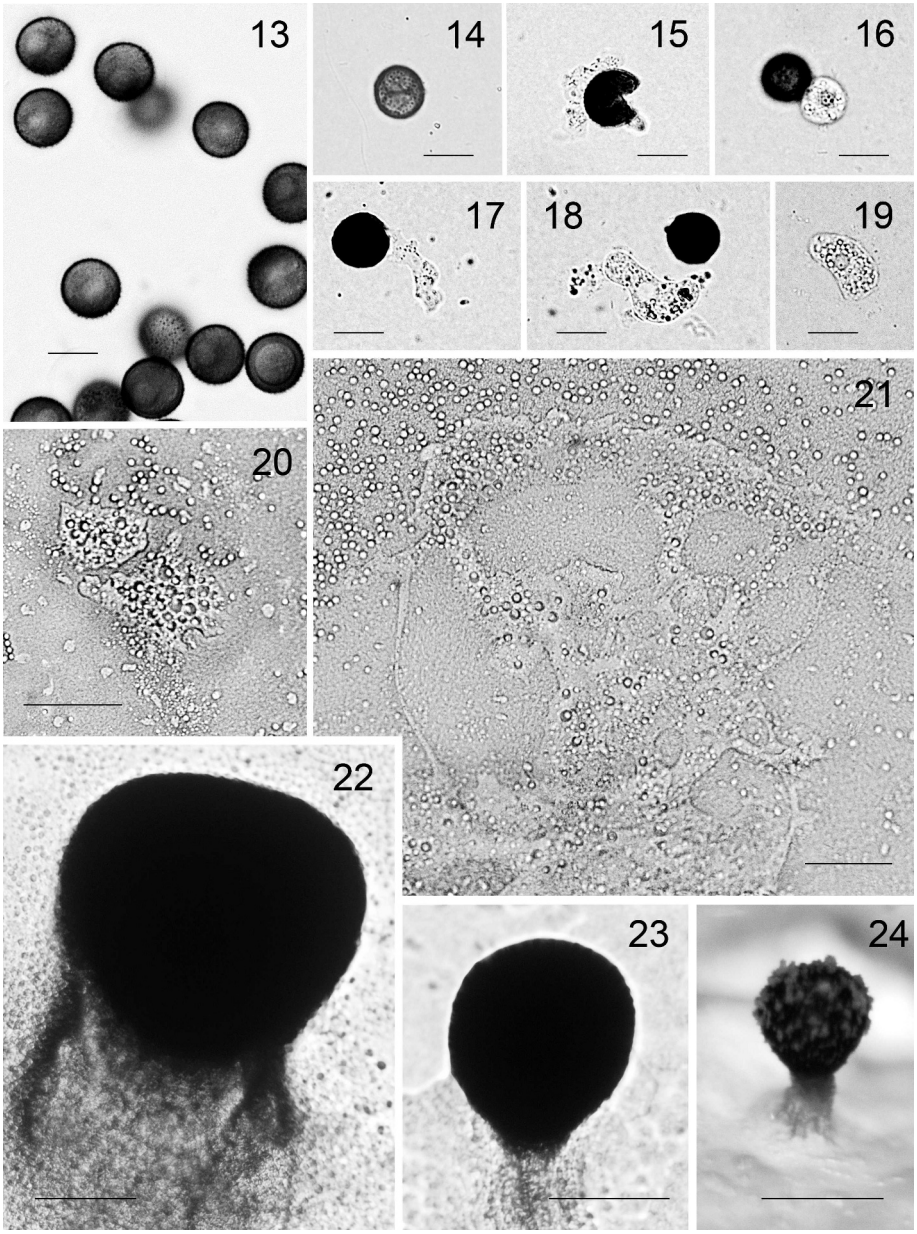


FIGS. 6–12. *Didymium infundibuliforme* by SEM. 6–8. Spores from different collections showing variable intensity of verrucae interconnected by an irregular net of bands . 6. (dwb 2845). Bar = 10 µm. 7. (Lado 15611). Bar = 10 µm. 8. (dwb 2825). Bar = 10 µm. 9. Capillitium (dwb 2834). Bar = 50 µm. 10. Detail of capillitial threads (dwb 2851). Bar = 10 µm. 11. Detail of spore ornamentation (Lado 15611). Bar = 5 µm. 12. Detail of spore ornamentation (dwb 2851). Bar = 1 µm.

Albardón, 31°23′07″S 68°35′41″W, 830 m, 6-VI-2007, on dead leaf bases of *Puya* sp. in moist chamber culture (pH 7.1), dwb 2927. CHILE. I Region, Tarapacá: Parinacota, Putre, Zapahuira, km 95 Road Ch-11, 18°20′35″S 69°30′53″W, 3322 m, 14-I-2004, on remains of *Eulychnia iquiquensis*, Lado 15559 (MA-Fungi 78326). II Region, Antofagasta: Taltal, Mina Liverpool, km 103 of Road Ch-B710, 25°00′19″S 70°24′23″W, 935 m, 21-I-2004, on remains of *Copiapoa* sp. Lado 15611 (MA-Fungi 78327). Taltal, Paposo, km 89 of Coastal Road, 24°53′45″S 70°31′29″W, 60 m, 21-I-2004, on remains of *Copiapoa* sp., Lado 15616 (MA-Fungi 78328). Caleta Colorada, 49 km from Paposo, 24°41′53″S 70°33′43″W, 36 m, 9-V-2008, on dead leaf bases of *Puya* sp. in moist chamber culture (pH 6.3), dwb 3044; 19-V-2008, (pH 6.7), dwb 3060; 25-V-2008, (pH 6.3), dwb 3056. III Region, Atacama: Chañaral, Pan de Azucar National Park, mirador Pan de Azucar, 26°06′40″S 70°38′54″W, 313 m, 21-V-2008, on dead leaf bases of *Puya* sp. in moist chamber culture (pH 6.5), dwb 3057.

Etymology. From the Latin *infundibulum* (funnel) + *formis* (form): referring to the shape of the sporocarp.

Habitat. On dead leaf bases of the bromeliad *Puya*

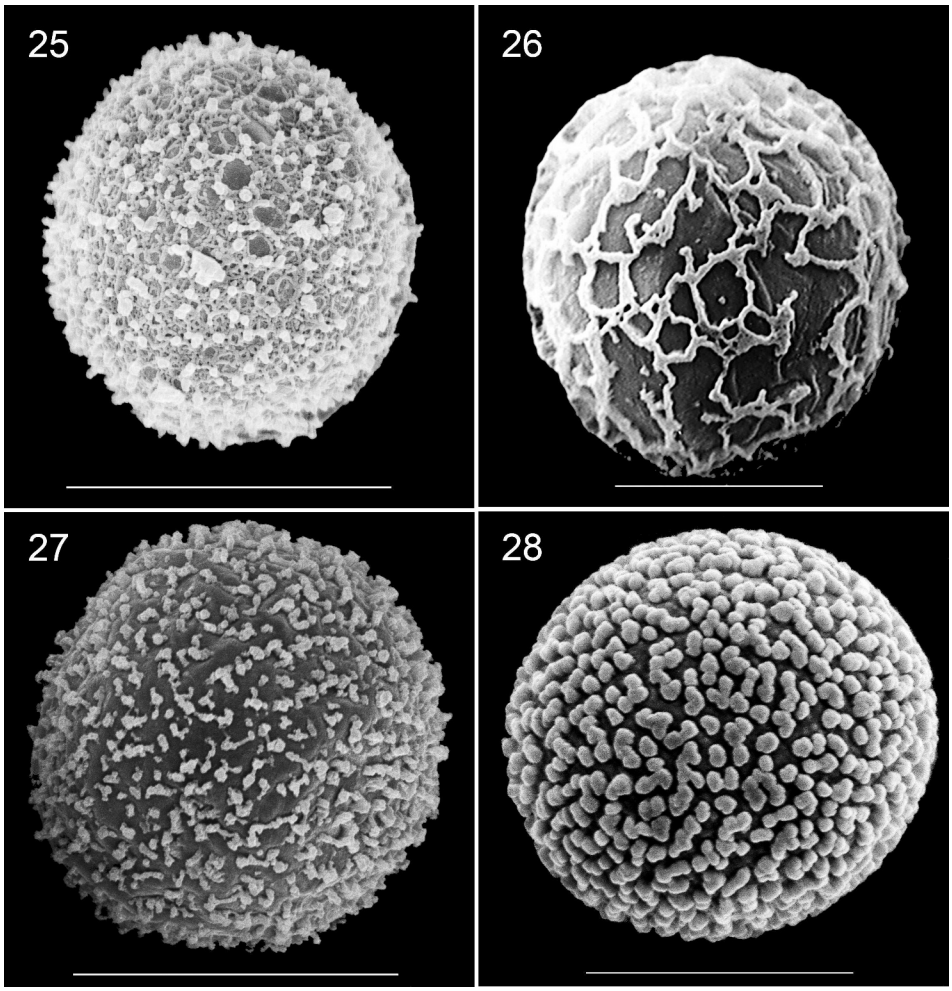


FIGS. 13–24. *Didymium infundibuliforme*. Life cycle. 13. Spores in a culture slide before germination begins (dwb 2834). Bar = 10 μm. 14–19 (dwb 2825). Bar = 10 μm. 14. Spore with two protoplasts just before germination. 15. Germinating spore showing V-shape split in the spore wall and myxamoeba. 16. Spore with microcyst. 17–18. Spores with myxamoebae. 19. Myxamoeba. 20. Two early reticulate plasmodia (dwb 2834). Bar = 100 μm. 21. Hyaline phaneroplasmodium (dwb 2834). Bar = 100 μm. 22–23 (dwb 2834). Developing sporocarps. 22. Bar = 100 μm. 23. Bar = 200 μm. 24. Maturing sporocarp showing lime crystal deposits (dwb 2834). Bar = 250 μm.

spp. and on dead remains of the cacti *Copiapoa* sp. and *Eulychnia* sp.

Distribution. Known from northwestern Argentina (Jujuy, Catamarca, La Rioja and San Juan states) and northern Chile (Antofagasta, Atacama and Tarapacá regions). Possibly occurring in other areas of South America, following the distribution of species of the plant genus *Puya* or certain cacti.

Life cycle in agar culture.—Germination of the spores was by a V-shape split in the spore wall, starting in the thinner area of the spore wall (FIGS. 15–17). Some spores in each quadrant (isolate dwb 07110 from dwb 2825) had germinated 16 h after being placed on 0.75% water agar (WA). In other plates germination took more than 48 h. The spores produced myxamoebae about 15–20 μm long (FIGS. 17–19), some of



FIGS. 25–28. Spores of different species of Didymium as viewed by SEM. 25. *Didymium infundibuliforme* (dwb 2851). Bar = 10 μm. 26. *Didymium subreticulosporum* (MA-Fungi 40475). Bar = 5 μm. 27. *Didymium umbilicatum* (Lado 11206). Bar = 10 μm. 28. *Didymium wildpretii* (EC-AET 422). Bar = 5 μm.

which began to retract pseudopodia and differentiate into microcysts almost immediately (FIG. 16). Swarm cells were not observed, possibly due to the presence of too little surface water, which is necessary for the production of swarm cells in some species (Haskins and Wrigley de Basanta 2008). After a few days small agar blocks, containing myxamoebae and some spores and spore cases, were transferred from the germination plate to fresh plates with 1.5% WA with the bacterial melange isolated incidentally with the spores. The population of amoebae grew rapidly in these plates and sterile Quaker oat flour was added to supplement the food supply. Before the amoebal population growth began to slow (after 12–15 d), small agar blocks with amoebae were transferred to half strength cornmeal agar (CM/2) plates and fed again with sterile Quaker oat flour. After a further 10–12 d small hyaline young plasmodia were observed among many microcysts and the rich bacterial melange that had developed in the plate. These plasmodia resembled protoplasmodia and exhibited slow intermittent cyclosis. They ingested myxomycete microcysts, which can be observed in food vacuoles inside the plasmodia (FIG. 20) and were seen circulating inside the vacuoles as digestion occurred. Sterile Quaker oat flour was added and the parafilm seal used on the dishes was not replaced, thereby altering the gas composition in the culture. The plasmodia grew from hyaline reticulate forms (FIG. 20) with irregular cyclosis to larger milky-colored phaneroplasmodia (FIG. 21) with normal cytoplasmic streaming over a further 8–10 d. The first sporocarps were observed 40–50 d from sowing the spores, darkening as the spores matured, to become black and shiny (FIGS. 22–23). The lengthening stalk and circular hypothallus were observed on the agar as soon as the spores darkened. Later the funnel-shape, typical sporocarps were seen and spots

of lime crystals began to appear on the surface of the sporotheca. Small pieces of agar with the sporocarps still immature were transferred to sterile 2% WA to mature and then dried slowly with the lid on (FIG. 24). Material obtained by means of agar culture had sporocarps, capillitium and spores identical to those observed in the moist chamber collection from which it was isolated.

DISCUSSION

Didymium infundibuliforme is a distinctive myxomycete. The characters that make this species unique in the genus are the funnel-shape sporocarps with white stalks and the apical circumscissile dehiscence of the sporotheca, which leaves a calyculus-like base. In addition, using the terminology proposed by Rammeloo (1974), the spores are ornamented with verrucae and bacula interconnected by an irregularly meshed net of bands on the epispore (FIGS. 11–12). The characteristic invagination of the peridium, reaching the base of the sporotheca, follows the funnel shape of the sporocarp, with the capillitium and spores located in the space created by the infolding peridium. This can be observed best with a stereomicroscope in mature dehisced sporocarps (FIG. 4), but the invagination of the peridium is also visible in mounted specimens as part of the circular dehisced ''lid'' (FIG. 5).

Other species of this genus, which are morphologically close to *Didymium infundibuliforme* and have been described from arid areas, are *D. umbilicatum* D. Wrigley, Lado et Estrada, *D. subreticulosporum* Oltra, G. Moreno & Illana and *D. wildpretii* Mosquera, Estrada, Beltrán-Tej., D. Wrigley & Lado. The closest species appears to be *D. umbilicatum*, but the latter differs in producing flattened discoid sporocarps to short plasmodiocarps versus the funnel-shape sporocarp of *D. infundibuliforme*, the irregular versus circumcissile dehiscence, the short stalk, which sometimes is limited to the restricted base of the sporotheca versus the defined calcareous stalk of *D. infundibuliforme*, and the different spore ornamentation (Wrigley de Basanta et al 2008). Spore ornamentation of *D. umbilicatum* consists of warts fused in an irregular subreticulum (FIG. 27), whereas in *D. infundibuliforme* the warts have a network of bands interconnecting their bases on the surface of the spore (FIGS. 6–8, 11–12, 25). In addition the peridium of *D. umbilicatum* is yellow-brown to brownish pink and that of *D infundibuliforme* is colorless.

Didymium subreticulosporum has globose to reniform sporocarps. The sporotheca occurs on a dark and longer stalk (0.2–0.9 mm) instead of being funnel-shape on a short stalk (0–0.4–0.3 mm), as is the case of the new species, and the former has no true capillitium versus the abundant capillitium of *D. infundibuliforme*. *Didymium subreticulosporum* also has smaller spores, with a reticulum that is broken on one side (Moreno et al 1996:57, Mosquera et al 2000:980) (FIG. 26), versus the ornamentation of interconnected verrucae and bacula in *D. infundibuliforme*.

Didymium wildpretii has a reduced or absent columella but its yellow sporotheca versus white in this species and the small spores (7.5–9.5 μm diam vs. 12–13 μm), which are covered with warts that are dense, flat and uniform (Lado et al 2007b) (FIG. 28) not with verrucae and bacula with a network of bands interconnecting their bases, differentiate it from *D. infundibuliforme*. In addition *D. infundibuliforme* has none of the enlargements on the capillitium that are characteristic of *D. wildpretii*. We outlined the characters that distinguish these species (TABLE I).

Blackwell and Gilbertson (1980) described *Didymium eremophilum* M. Blackw. & Gilb. from the Sonoran Desert in Arizona, but this species lacks a capillitium and has tall, stalked sporocarps and smaller spores, 9–11 μm diam, that are echinulate. *Didymium applanatum* Nann.-Bremek. has a stalk containing lime and also deshisces ''by a more or less circumcissile crack'' (Nannenga-Bremekamp 1972), but its grouped, discoid sporocarps with a wide umbilicus below the sporotheca and a pale brown basal disk distinguish it from *D. infundibuliforme*. The former also has smaller spores (8–11 μm diam vs. 12–13 μm diam) with ''small warts mixed with groups of larger ones'' (Nannenga-Bremekamp 1972) and not verrucae and bacula interconnected at their bases by an irregularly meshed net of bands as in *D. infundibuliforme*. *Didymium circumscissile* K.D. Witney & L.S. Olive has a circumscissile dehiscence which leaves a deep cup, but its sporocarps are sessile or on a restricted base and are globose to turbinate or occasionally reniform (Whitney and Olive 1983) and not funnel-shape with a peridial invagination from the surface to the base of the sporotheca and stalked such as *Didymium infundibuliforme*. In addition the former species usually lacks a capillitium and has spores that are covered with minute warts not ornamented with an interconnected net of verrucae and bacula as is the case of *D. infundibuliforme*.

The sporocarps of *D. squamulosum* (Alb. & Scwein.) Fr. have white stalks filled with lime, but this species also has a sporotheca that is subglobose to oblate and a calcareous columella not a funnel-shape sporotheca with no true columella such as *D. infundibuliforme*. Apart from this *D. squamulosum* spores are smaller (8–11 μm diam vs. 12–13 μm diam) and covered with spines or warts, not interconnected at their bases by an irregularly meshed net of bands as is the case for the spores of *D. infundibuliforme*.

TABLE I. Distinguishing characters of some recently described species of *Didymium*

Species	Sporophores	Stalk	Peridium	Capillitium	Columella	Spore size	Spore by SEM
D. infundibuliforme SEM FIG. 25	Funnel-shape sporocarps, 0.2–0.6 mm light, gray to white, stalked	White	Colorless, invagination reaching base of sporotheca, dehiscence circumscissile.	Straight or undulating, uniform diameter, abundant cross connections	Absent	12–13 μm	verrucae and bacula with a network of bands interconnecting their bases
D. subreticulosporum SEM FIG. 26	Globose to reniform sporocarps, 1–2 mm tall, white to gray, stalked	Black	Light orange-yellow, formed of platelets, dehiscence irregular	No true capillitium	Present, enlarged and irregular	8.4–11.6 μm	Reticulum of bands, broken on one side
D. umbilicatum SEM FIG. 27	Flattened sporocarps to sub-plasmodiocarps, umbilicate above, 0.15–0.4 mm tall, pale gray to white, sessile to short stalked	Yellow-brown to brownish-pink when present	Yellow-brown to brownish-pink, depressed into an umbilicus above, dehiscence irregular	Undulating, uniform diameter, few cross connections	Absent	11–14 μm	Warts fused in a single layer, discontinuous subreticulum
D. wildpretii SEM FIG. 28	Subhemispherical to reniform sporocarps or plasmodiocarps, 0.1–0.75 mm tall, yellow, stalked to sessile	Orange-yellow when present	Iridescent, without platelets or invagination, dehiscence irregular	Rigid and scarcely branched, vesicular enlargements on the capillitial tubes.	Absent or reduced	7.5–9.5 μm	Densely uniformly warted. Some warts fused in a subreticulum

The fastest germination of *Didymium infundibuliforme* observed in agar culture was 16 h (isolate 07110 from dwb 2825). Spores produced two amoebae from each spore, and two protoplasts could be observed in spores just prior to germination (FIG. 14) that were not there before (FIG. 13). Other plates prepared from the same collection took up to 4 d. Another germination chamber (isolate 0806 from dwb 2834) had no germination at 48 h but 13 d later was full of amoebae and microcysts. No observations could be made during this interval.

Didymium umbilicatum, described by Wrigley de Basanta et al (2008), and the succulenticolous species of the same genus, *Didymium wildpretii* (Lado et al 2007b), both required fewer than 48 h to germinate on 0.75% water agar. Spores from *Didymium annulisporum* were reported (Keller and Schoknecht 1989) to take about 19 h to germinate in hanging drops and some isolates had nearly 100% germination at 48 h. The life cycle on agar of *Didymium infundibuliforme* from soaking the spores to the formation of sporocarps was 41 d (isolate from dwb 2825) to 50 d (isolates from dwb 2834). Another species of the genus from arid areas, *D. eremophilum*, completed its life cycle on agar in 5 d (Blackwell and Gilbertson 1980), but the life cycle of *D. umbilicatum* on agar required at least 51 d (Wrigley de Basanta et al 2008) and that of *Didymium wildpretii* was 28–56 d (Lado et al 2007b). Life cycles on artificial media vary with the culture conditions, as cited by Everhart and Keller (2008). In the case of the culture of *D. infundibuliforme* it is possible that the natural bacterial melange isolated with the spores was insufficient to support a faster germination or a shorter life cycle because no additional bacteria were supplied. The length of the life cycle under natural conditions in arid environments, as suggested for *D. umbilicatum* (Wrigley de Basanta et al 2008), also might be affected by the frequent production of microcysts or sclerotia. Slow drying of the developing sporocarps seems to be necessary because in culture if they were allowed to dry too quickly they became wrinkled and misshapen.

Didymium infundibuliforme also produced small hyaline phaneroplasmodia from protoplasmodia (FIG. 20) and the former grew to form fine reticulate veins (FIG. 21) as noted in the culture of *D. umbilicatum* (Wrigley de Basanta et al 2008). The mature plasmodia observed in agar culture were small (0.2–1.5 mm) and gave rise to a single or only a few sporocarps each. This might account for the dispersed habit of this species and is probably another strategy for success in arid areas as Blackwell and Gilbertson (1980) noted for *D. eremophilum*, which also produced tiny young plasmodia after 72 h and then small phaneroplasmodia. Whitney and Olive (1983) observed that plasmodial size of *D. circumscissile* depended on culture conditions such as food supply, and this was supported by observations made by Keller and Schoknecht (1989) while culturing *D. annulisporum*. However *Didymium infundibuliforme* also produced small plasmodia in moist chamber culture (FIG. 1), but again this might have been limited by the quantity of the food organisms even though it was growing on its natural substrate.

Didymium infundibuliforme has been found primarily on the dead leaf bases of *Puya* spp. This genus, native to South America, is an evergreen perennial that is a member of the Bromeliaceae. The plants form large, dense rosettes of gray-green leaves, with sharp spines along the edge. In the field specimens *D. infundibuliforme* was found on the upper surfaces at the base of the leaves that form the rosettes, where some small amount of moisture was retained. In moist chamber culture of the *Puya* leaf bases the new species appeared after 2–33 d with a mean incubation time of 11 d. This much shorter incubation in moist chamber culture compared to spore to spore culture is probably because the species exists in the form of sclerotia or microcysts on the substrate, as suggested by Wrigley de Basanta et al (2008). The small plasmodia were observed on the leaf surface in the cultures (FIG. 1), but they did not migrate far and formed fruiting bodies within a few hours to 2 d. From 35 moist chamber cultures of this substrate the new species appeared in 14 (40%). The pH of the substrates producing this species in moist chamber culture was 6.3–7.2 with a mean 6.8, conditions similar to those of the moist chamber cultures that produced another species, *D. umbilicatum* (Wrigley de Basanta et al 2008).

Genus *Didymium* seems to be well adapted to arid areas, where it has accounted for approximately 20% of the species found in some studies (Lado et al 2007a, Estrada-Torres et al 2009). In addition an increasing number of species of *Didymium* have been described from arid areas (Blackwell and Gilbertson 1980, Lizáraga et al 1996, Novozhilov and Zemlyanskaya 2006, Lado et al 2007b, Estrada-Torres et al 2009, Wrigley de Basanta et al 2008). Some of these have small but significant morphological differences as discussed above and possibly physiological differences that determine the specific niche of each. One possible explanation for the variation in the genus in arid habitats is that the geographical isolation of islands of vegetation in deserts might have lead to reproductive isolation, perhaps enhanced by ultraviolet radiation increasing the rates of mutation, thus the rate of speciation. Long-term isolation and a mixture of heterothallic and nonheterothallic reproductive systems were suggested by Clark and Mires

(1999) as being sufficient to produce the many morphological variations and subsequent taxonomic difficulties in the long-stalked species of *Didymium*. In addition ElHage et al (2000), who carried out a biosystematic study of *Didymium squamulosum*, showed that some morphospecies actually consisted of a species complex with a number of biological species involved. These authors suggest that some morphologically similar species might belong to such a species complex and be local variants of it. To determine the relationships among the morphospecies from arid areas more culture work and a thorough examination of the reproductive systems in this group is needed. The taxonomic and evolutionary relationships within the genus perhaps will become clearer with a monographic analysis of the species of *Didymium* and when DNA work on the group is perfected.

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