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Spore to spore culture of *Didymium operculatum*, a new Myxomycete from the Atacama Desert of Chile

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Abstract: A new species of *Didymium* (Myxomycetes), *D. operculatum*, is described in this paper, and details of its life cycle are provided. The new species was recorded during studies of the Atacama Desert in Chile. It has been collected directly in the field and isolated in moist chamber cultures prepared with material from an endemic cactus. The distinguishing characters of this species are its dehiscence by means of an apical operculum combined with a whitish calcareous stalk and the banded reticulate ornamentation of the spores. The morphology of this new myxomycete was examined with scanning electron microscopy and light microscopy, and micrographs of relevant details are included in this paper. Some comments are made on the patterns of distribution of *Didymium* species in arid lands and adaptive characters enabling this genus to colonize such extreme environments. It is proposed that a longer cycle and the ability to resort to resistant forms many times during their development reflect the response of these myxomycetes to the largely unfavorable conditions of their environment.

Key words: agar culture, Amoebozoa, *Copiapoa*, Eumycetozoa, morphogenesis, SEM, taxonomy, xeric

INTRODUCTION

Studies of the myxomycetes from arid areas of the Neotropics (Lado et al. 2007a, 2009, in press; Estrada-Torres et al. 2009; Wrigley de Basanta et al. 2010) have included a series of intense surveys in different countries. During one of these surveys in Chile a new species of genus *Didymium* was found in the Atacama Desert. Schrader (1797) described the myxomycete genus *Didymium* based on eight species, of which only *Didymium farinaceum* Shrad., now synonymous with *Didymium melanospermum* (Pers.) T. Macbr. and considered to be the type species, remains included in the genus as it is known today (Martin and Alexopoulos 1969, Lado 2005–2010). The genus now includes approximately 70 species, many (~ 25%) described in the past 20 y (Lado 2005–2010), particularly species from arid areas of the world (Blackwell and Gilbertson 1980; Lizárraga et al. 1996; Novozhilov and Zemlyanskaya 2006; Estrada-Torres et al. 2009; Wrigley de Basanta et al. 2008, 2009). Even though some of the areas sampled for these papers have not been systematically studied for myxomycetes until recently, the number of *Didymium* species recorded from xeric environments, almost a quarter of all species reported in some studies (Lado et al. 2007, Estrada-Torres et al. 2009), show that the genus is especially well adapted to arid areas. In the recent research of northern Argentina and Chile, including part of the Atacama Desert, a small myxomycete was isolated from moist chamber cultures and also collected in the field that did not correspond to any described species. It was cultured from spore to spore on agar and maintained its morphological characters and thus is described here as a new species.

MATERIALS AND METHODS

The study area was at the southern limit of the Atacama Desert, 23–30°S, 68–71°W. This is one of the driest places on earth with areas that receive only 1 mm rain per annum, and in places only a sea mist, the ''camanchaca'', provides water sufficient to keep some of the plants alive. The vegetation of the study area was predominantly xerophilous scrubland with endemic species of cacti such as the globose *Copiapoa* spp. and the candelabra *Eulychnia* spp.

Sampling was done Feb 2008, and known or suspected habitats of myxomycetes were examined in the field. Collections were made also of the dead remains of various plants for use as substrates in the moist chamber culture of myxomycetes. 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 the localities were geo-referenced with a GPS (Magellan eXplorist 600 5.1, Datum WGS84). Moist chamber cultures were set up as described by Wrigley de Basanta et al. (2009). All sporocarps of the same species in one culture were regarded as representing one collection. Agar cultures were prepared with spores from mature sporocarps of collections dwb 3142 from moist chamber (mc) and Lado 19080 from a field collection (fc). Spores were sown on 0.75% water agar (WA)

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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 maintained in an incubator at 22 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). Germination slide cultures were set up as described by Spiegel et al. (2005).

All specimens are deposited in the herbaria MA-Fungi (sub Lado), TLXM (sub ET) and the private collection of the first author (dwb). Microscope measurements and observations were made with material mounted directly in Hoyer's medium. A differential interference contrast (DIC) microscope was used to obtain descriptive data and light micrographs. Spore measurements were made of 10 spores from each of the collections. The critical-point drying technique 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, with 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 (Anon. 1976).

RESULTS

Didymium operculatum D. Wrigley, Lado et Estrada, sp. nov.

MycoBank MB519191

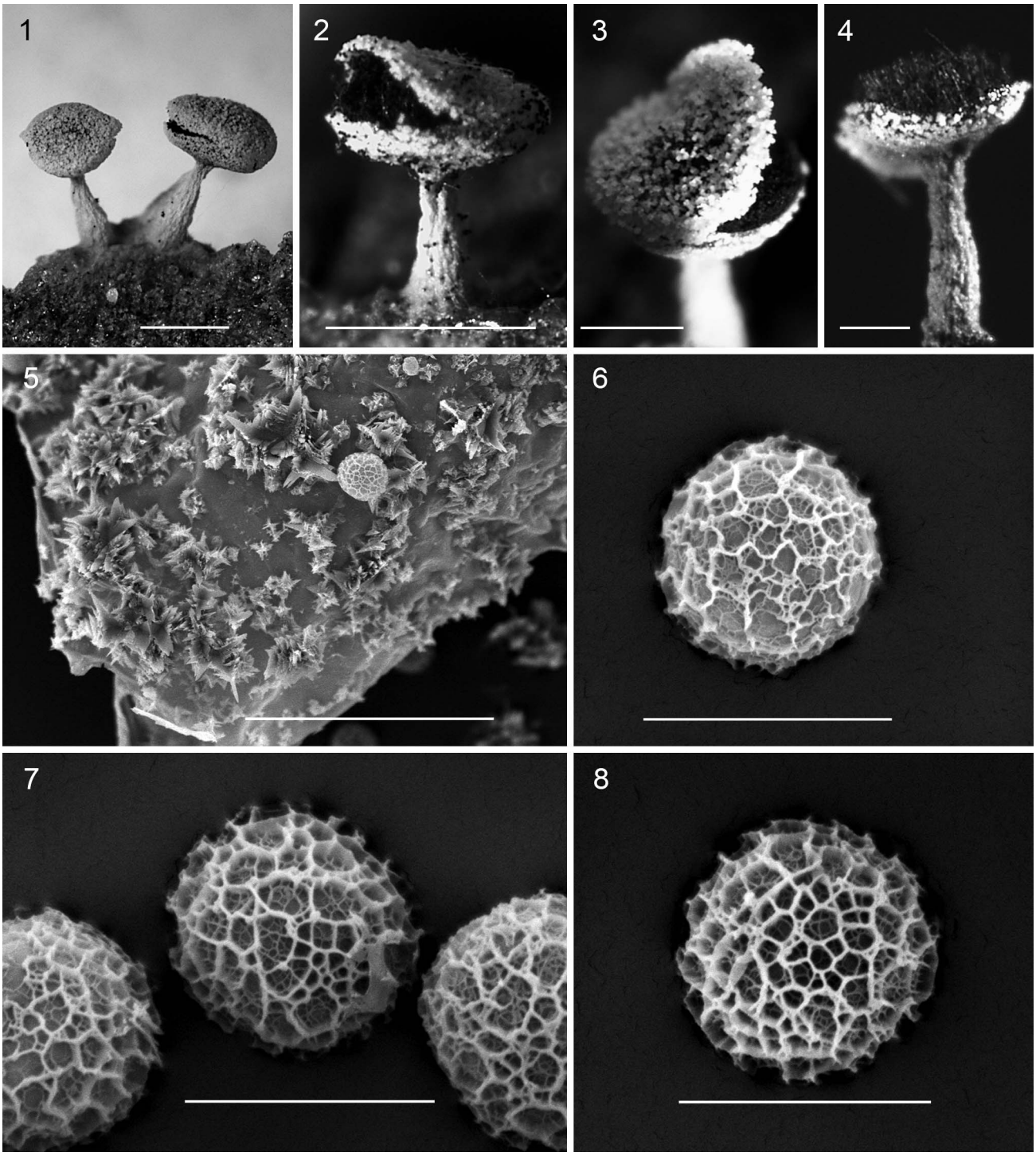
Sporophora sporocarpica, dispersa vel aggregata. Sporocarpia 0.3–0.9 mm alta, stipitata. Sporotheca disciformis, (0.2–) 0.3–0.6 (–0.8) × 0.17–0.4 mm, pallidissime grisea vel prorsus alba. Stipes calcareus, erectus, 0.1–0.56 mm altus, subconicus. Peridium unicum, membranaceum, crystallis albis, calcareis, coopertum. Dehiscentia circumscissa. Sine columella. Capillitium filamentosum, filamentis 0.5–2 mm diam, parce ramificatis, erectis vel undulatis. Sporae liberae, griseobrunneae, (9.5–)10–11(–12.5) mm diam, globosae, reticulatae.

Sporophores sporocarpic, dispersed, rarely grouped. Sporocarps (FIGS. 1–4, 21), 0.3–0.9 mm high, stipitate. Sporotheca discoid from above, elliptical to oblate from the side, occasionally obconical, (0.2–)0.3–0.6(–0.8) × 0.17–0.4 mm, with a lid (FIGS. 2–4), covered with calcium carbonate crystals, light gray (264. l. Gray) to white (263. White), especially above, iridescent below where the crystals are sparse or absent. Stalk erect, conical to subcylindrical, attenuated toward the sporotheca, 0.1–0.56 mm high, 100–300 μm wide at the base, 70–100 μm at the apex, calcareous, with lime crystals inside and on the surface, ending at the base of the sporotheca, yellowish white (92. y White) to pale yellow (89. p. Y) to grayish yellow (90. gy. Y) in reflected light, mid orange-yellow to grayish yellow (71. m. OY-90. gy. Y-40. s. r B) by LM. Hypothallus inconspicuous or absent, when present, small, irregular, reddish brown and membranous. Peridium single, membranous, colorless to light grayish brown (79. l. gy. y Br) by LM, its inner surface smooth, also by SEM, covered with abundant to scattered, white, stellate, lime crystals, some the size of the spores (FIGS. 1, 5), often absent below showing the iridescent peridium; dehiscence circumscissile, the apex of the sporotheca dehiscing completely in the form of a lid (FIGS. 2–4) leaving a broad shallow base. Columella absent. Capillitium filiform, attached to the base of the sporotheca, threads 0.5–2 μm diam, branched, straight to undulating, with cross connections, light gray brown (60. l. gy. Br) to colorless, smooth. Spores free, black in mass, mid-brown (58. m. Br) to grayish brown (61. gy. Br-80. gy. y Br) by LM, globose, (9.5–)10–11(–12.5) μm diam, reticulate under LM (FIGS. 9, 10), banded reticulate, with 9–12 meshes across the hemisphere, and with a second reticulum underneath, visible through the meshes by SEM (FIGS. 6–8). Plasmodium a phaneroplasmodium, translucent, to milky, to brownish by transmitted light (FIGS. 14–17).

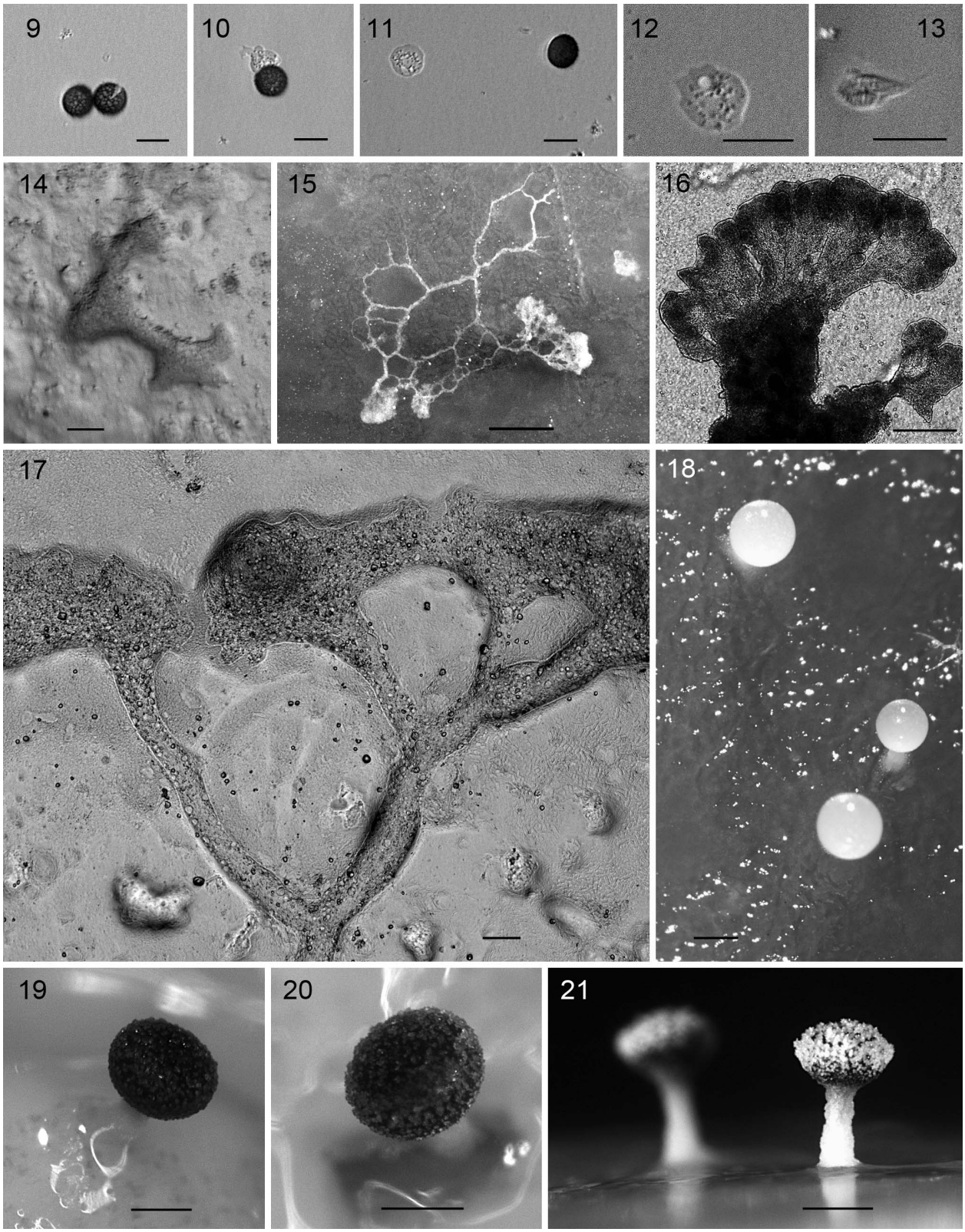
Holotype. CHILE. Region II Antofagasta: Antofagasta, route RN-1, Blanco Encalada, 24°25′57″S, 70°31′59″W, 137 m, 28-II-2009, on epidermis of dead *Copiapoa* sp. in moist chamber culture (pH 7.1), *D. Wrigley de Basanta*, dwb 3142 (MA-Fungi 74050).

Specimens examined. CHILE. Region II Antofagasta: Antofagasta, route RN-1, Caleta Colorada, 49 km north of Paposo, 24°41′53″S, 70°33′43″W, 36 m, 18-II-2008, on dead remains of *Copiapoa* sp., Lado 19081, Lado 19082, Lado 19083, Lado 19084. Ibidem, on agar culture of spores from Lado 19080, 3-V-2010, isolates dwb 1021 and dwb 1023. Antofagasta, route RN-1, El Cobre, Lilian mine, 24°17′50″S, 70°29′25″W, 804 m, 8-XI-2010, on dead remains of internal tissues of *Copiapoa solaris*, (mc, pH 8.3), ET-12157, (mc, pH 7.8), ET-12160. Antofagasta, route RN-1, Blanco Encalada, 24°25′57″S, 70°31′59″W, 137 m, 7-III-2009, on epidermis of dead *Copiapoa* sp. (mc, pH 7.1), dwb 3155; 28-II-2009, (mc, pH 6.3), dwb 3157; 28-X-2010, on dead remains of internal tissues of *Copiapoa* sp. (mc, pH 8.8), ET- 12148; 5-XI-2010 (mc, pH 9.0), ET-12152; (mc, pH 8.8), ET-12153. Ibidem, on agar culture of spores from the type specimen dwb 3142, 27-IV-2009, isolate dwb 0918. Region III Atacama: Chañaral, km 12 road to Pan de Azúcar National Park, 26°16′26″S, 70°39′23″W, 20 m, 26-X-2010, on dead remains of internal tissue of *Copiapoa cinerascens* (mc, pH 9.0), ET-12142, (mc, pH 9.2), ET-12143; 28-X-2010 (mc, pH 9.4), ET-12147; 8-XI-2010, (mc pH 9.0), ET-12162.

Other material examined. *Didymium infundibuliforme* D. Wrigley, Lado & Estrada, 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., MA-Fungi 78320 [Holotype]. CHILE. Region II Antofagasta: Taltal, Mina Liverpool, km 103 of road Ch-B 710, 25°00′19″S, 70°24′23″W, 935 m, 21-I-2004, on remains of *Copiapoa* sp., MA-Fungi 78327. Caleta Colorada, 49 km north of Paposo, 24°41′53″S, 70°33′43″W, 36 m, 9-V-2008, on dead leaf bases



FIGS. 1–8. 1–5. *Didymium operculatum* (Holotype dwb 3142, MA-Fungi 74050). 1. Habit. Bar = 0.5 mm. 2. Sporocarp showing circumscissile dehiscence, with lime crystals on peridial surface. Bar = 0.5 mm. 3. Detail of sporotheca with operculum lifting off. Bar = 0.2 mm. 4. Dehisced sporocarp showing capillitium and spore mass after the operculum has lifted off. Bar = 0.2 mm. 5. Detail of peridium showing crystals by SEM. Bar = 50 μm. 6. *Didymium operculatum* (isolate dwb 0918). Spore from agar culture by SEM. Bar = 10 μm. 7–8. *Didymium operculatum* (Holotype, dwb 3142, MA-Fungi 74050). 7. Group of spores by SEM. Bar = 10 μm. 8. Spore by SEM showing banded reticulate ornamentation with a second reticulum beneath the outer one. Bar = 10 μm.



FIGS. 9–21. *Didymium operculatum*. Stages in the life cycle. 9–13. Slide culture (Lado 19080) by light microscope with DIC. Bar = 10 µm. 9. Germination V-shaped slit in spore. 10. Flow of protoplasm through opening in the spore. 11. Amoeba. 12. Amoeba showing contractile vacuole. 13. Flagellate swarm cell. 14. Early plasmodium on agar (isolate dwb 0918). Bar = 100 µm. 15. Milky white phaneroplasmodium with network of very trailing veins (isolate dwb 0918). Bar = 1 mm. 16. Older

of *Puya* sp. (mc, pH 6.3), dwb 3044. *Didymium umbilicatum* D. Wrigley, Lado & Estrada, MEXICO. Querétaro, Peñamiller, Plazuela, 21°03′24″N, 99°42′35″W, 1395 m, on bark of *Yucca* sp. in mc, 19-III-2005, dwb 2554, MA-Fungi 73566 [Holotype]. *Didymium reticulosporum* Novozh. & Zemly., RUSSIA. Volgograd province, Otrada wash, Ergeni, the watershed of the Volga River, 48°34′43″N, 44°21′04″E, 100 m, on dead twigs of *Artemisia lerchiana* in moist chamber culture, 24.07.2001, leg. I.V. Zemlianskaia, det. Yu K. Novozhilov, LE 220334 [type material].

Etymology. From the Latin *operculatus* (with a lid) referring to the dehiscence of the sporocarp.

Habitat. On the dead remains of the cactus *Copiapoa* spp.

Distribution. Known only from northern Chile (Antofagasta and Atacama).

Life cycle in agar culture.—Spores germinated by a V-shape split in the spore wall (FIG. 9). Some spores in two quadrants (isolates dwb 0918, from dwb 3142) had germinated 48 h after being placed on 0.75% water agar (WA). Spores from one field collection (isolate dwb 1008, from Lado 19080) took up to 4 d for germination to occur. The spores produced myxamoebae about 10 μm long (FIGS.10, 12), and flagellate swarm cells 10–12 μm long (FIG. 13). 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 mélange isolated incidentally with the spores. The population of amoebae grew rapidly in these plates. During the log growth phase of the amoeba population (after 15–20 d) small agar blocks with amoebae were transferred to half strength cornmeal agar (CM/2) plates. After a further 10–15 d small hyaline plasmodia were observed among many microcysts and the rich bacterial mélange that had developed in the plate. These plasmodia resembled protoplasmodia and exhibited slow intermittent cyclosis. They ingested myxomycete microcysts, and amoebae that can be observed in food vacuoles inside the plasmodia (FIG. 14), and were seen circulating inside the vacuoles as digestion occurred. The hyaline plasmodia grew, with irregular cyclosis, to larger milky phaneroplasmodia (FIG. 15), with normal reversible cytoplasmic streaming, then darkened to brownish in reflected light (FIGS. 16, 17) and produced sporocarps. The sporocarps were observed 40–102 d from sowing the spores. Early sporocarps were pale whitish yellow (FIG. 18), darkening as the spores matured to form an almost black sporotheca covered with spots of forming lime crystals, with a white stipe (FIGS. 19, 20). The agar plates with the sporocarps were dried slowly with the lid on until the sporocarps were mature (FIG. 21). Material obtained by means of agar culture had sporocarps, capillitium and spores identical to those observed in the moist chamber or field collection from which it was isolated.

DISCUSSION

Taxonomy.—Twenty collections of this new species have been obtained, five as field collections, 12 collections from moist chamber cultures and three from agar culture. The life cycle of this myxomycete has been observed on agar from spore to spore, both from spores produced by natural fruiting in the field and those produced by fruiting in moist chamber culture. This has confirmed the stability of its taxonomic characters. The characters that make this species unique in the genus are its dehiscence by means of an apical operculum combined with a whitish calcareous stalk and the banded reticulate ornamentation of the spores. This kind of spore ornamentation is rare in this genus. The only other member of genus *Didymium* with banded reticulate spores is *D. reticulosporum* Novozh. & Zemly., described by Novozhilov and Zemlyanskaya (2006). This species, from the steppe community of the Volga River basin, has subglobose to pulvinate sessile sporocarps, not discoid, stipitate sporocarps like *D. operculatum*. In addition *D. reticulosporum* was described as lacking a capillitium and its dehiscence is not by a circumscissile lid. The spores of *D. reticulosporum* are larger, 13–16 μm diam with 3–4 meshes across the hemisphere, whereas those of *D. operculatum* are 10–11 μm diam with 9–12 meshes across the hemisphere. The circumscissile dehiscence of *Didymium operculatum* by means of a lid or operculum is distinctive because the lids lift off and often can be found on the substrate near the sporocarp. *Didymium circumscissile* K.D. Whitney & L.S. Olive has a circumscissile dehiscence, but this leaves a deep cup, whereas the base left by *D. operculatum* after dehiscence is saucer-shaped (FIG. 4), and the sporocarps of the former species are sessile or on a restricted base and are globose to

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phaneroplasmodium on agar (isolate dwb 1012). Bar = 0.25 mm. 17. Leading edge of older phaneroplasmodium on agar (isolate dwb 0918). Bar = 50 μm. 18. Three early sporocarps forming on agar (isolate dwb 0918). Bar = 0.25 mm. 19–20. Sporocarps maturing on agar (isolate dwb 0918). Bar = 0.25 mm. 21. Mature sporocarps drying on agar (isolate dwb 0918). Bar = 0.25 mm.

turbinate or occasionally reniform (Whitney and Olive 1983) and not clearly stipitate, discoid sporocarps like *D. operculatum*. *Didymium circumscissile* also can be distinguished by its nearly smooth spores that are minutely and closely warted under oil immersion, not banded reticulate spores such as *D. operculatum*. The habit and general shape of some of the sporocarps of the new species are reminiscent of *D. infundibuliforme* D. Wrigley, Lado & Estrada, but this species has a clear funnel-shaped invagination of the peridium at the apex of the sporotheca, absent in *D. operculatum*, and warted spores by LM, not reticulate spores. *Didymium infundibuliforme* also has larger spores (12–13 µm diam vs. 10–11 µm diam) with different ornamentation by SEM (Wrigley de Basanta et al. 2009).

Some other species, similar in some aspect to the new species, are *Didymium squamulosum* (Alb. & Schwein.) Fr., *D. atrichum* Henney & Alexop. and *D. subreticulosporum* Oltra, G. Moreno & Illana. *Didymium squamulosum* has white calcareous stalks, but this species usually has a sporotheca that is subglobose to oblate and a calcareous columella, not a discoid sporotheca with no columella such as *D. operculatum*, although *D. squamulosum* is a variable species that sometimes can be discoid and lacks a prominent columella. Even in these cases however *D. squamulosum* spores are covered with spines or warts, not a banded-reticulum. *Didymium subreticulosporum* has spores with a broken reticulum but has globose to reniform sporothecae on dark noncalcareous stalks (Moreno et al. 1996, Oltra et al. 1997, Mosquera et al. 2000) and no true capillitium, whereas *D. operculatum* has discoid sporothecae on whitish calcareous stalks, abundant capillitium and banded reticulate spores. *Didymium atrichum* also has reticulate spores 10–11 µm diam, but the reticulum is only faintly visible under oil immersion, and by SEM the reticulum is denser with smaller mesh and without a second reticulum under the first. *Didymium atrichum* also has sessile, globose sporocarps to plasmodiocarps, with irregular dehiscence and practically no capillitium (Henney et al. 1980). On the other hand *D. operculatum* has discoid sporothecae on whitish calcareous stalks, abundant capillitium, circumscissile dehiscence and the spores are clearly reticulate under low magnification and are banded reticulate with a second reticulum below the first by SEM.

Culture.—In moist chamber cultures the new species took (7–)14–34(–51) d to produce mature sporocarps at room temperature (approx. 22 C), although it is not known whether these came from spores on the substrate or microcysts or small sclerotia. The pH of the cultures of internal tissue at 24 h was 7.8–9.4, similar to that of other cacti (Estrada-Torres et al. 2009). The pH of cultures of epidermal tissue was 6.3–7.1, somewhat neutral for cactus remains, but there was little inner tissue included in the material used for these moist chamber cultures, confirming that the epidermis of cacti have a more neutral pH (Estrada-Torres et al. 2009). The pH is similar to that of the substrates of *D. infundibuliforme* (Wrigley de Basanta et al. 2009) and *D. umbilicatum* (Wrigley de Basanta et al. 2009) in moist chamber culture. If the sporocarps dried too quickly some of them developed a depression on the surface of the lid. Several of the cultures produced more than 100 sporocarps, an unusually high number in this genus for a 9 cm culture dish, although only about 35% of the cultures of this substrate produced *Didymium operculatum*.

The whole cycle of *D. operculatum* in spore to spore culture on agar took a minimum of 40 d after inoculation of agar with spores until the first fruit bodies appeared. Whitney and Olive (1983) completed spore to spore culture of *D. circumscissile*, and the small phaneroplasmodia fruited 10 d after inoculation of hay infusion agar with spores. Another species that also had a much shorter life cycle on agar is *D. atrichum*. It germinated in hanging drops after 3–5 h and completed its life cycle in 7–14 d (Henney et al. 1980). In the case of *D. operculatum* most of the time was spent as amoebae, with cyst formation. Plasmodia were present only in the last 7–10 d. Spores from the field collection took longer to germinate (4 d vs. 2 d) and formed plasmodia 30 d after sowing the spores. These plates had plasmodia for more than 50 d before fruit bodies were formed, but the plasmodia formed sclerotia, even in the presence of bacteria and water, and only re-activated after transfer to fresh plates. The reason for this behavior is unknown, but this ability to avoid unfavorable conditions as sclerotia is of obvious advantage in the arid environment of its natural substrate. Similarly our observations have confirmed the ability of this myxomycete to frequently and rapidly form microcysts that excyst on transfer to a fresh plate, or on addition of food or water, when conditions become favorable again. This also was observed in agar culture of *D. wildpretii* (Lado et al. 2007b) and this species, described from arid areas of Spain and Mexico, also had a cycle that took up to 56 d. Two other *Didymium* species described from xeric environments had similar long life cycles on agar, *D. umbilicatum* (Wrigley de Basanta et al. 2008) a minimum of 51 d and *D. infundibuliforme* 41–50 d (Wrigley de Basanta et al. 2009). The environment in the agar cultures may not have been optimum for sporulation, but the incubation in moist chamber cultures of *D. operculatum* was up to 51 d on its natural substrate. Our hypothesis is that the longer

TABLE I. *Didymium* species described from arid environments

Species	Publication(s)	Substrate(s)	Country
D. eremophilum M. Blackw. & Gilb.	Blackwell and Gilbertson (1980)	*Carnegiea gigantea*	USA
	Estrada-Torres et al. (2009)	*Pachycereus weberi*	Mexico
D. infundibuliforme D. Wrigley, Lado & Estrada	Wrigley de Basanta et al. (2009)	*Puya* sp. *Copiapoa* sp.	Argentina
		Eulychnia sp.	Chile
D. mexicanum G. Moreno, Lizárraga & Illana	Lizárraga et al. (1996), Moreno et al. (1997)	*Agave schawii, Yucca* sp.	Mexico
	Lado et al. (In press)	*Echinopsis atacamensis*	Argentina
D. operculatum D. Wrigley, Lado & Estrada	This paper	*Copiapoa* sp.	Chile
D. reticulosporum Novozh. & Zemly.	Novozhilov and Zemlyanskaya (2006)	*Artemisia* sp.	Russia
D. subreticulosporum Oltra, G. Moreno & Illana	Oltra et al. (1997)	*Opuntia maxima*	Mexico
	Mosquera et al. (2000)		Spain
D. tehuacanense Estrada, D. Wrigley & Lado	Estrada-Torres et al. (2009)	*Agave* spp.	Mexico
D. umbilicatum D. Wrigley, Lado & Estrada	Wrigley de Basanta et al. (2008)	*Agavaceae*	Mexico
D. wildpretii Mosquera, Estrada, Beltrán-Tej., D. Wrigley & Lado	Lado et al. (2007b)	*Cactaceae* (11 species)	Mexico Spain
	Lado et al. (In press)	*Opuntia* spp.	Argentina

cycle reflects the response of these myxomycetes to the largely unfavorable conditions of their environment and their ability to resort to resistant forms many times during their life cycle. This is an alternative, but not contradictory, hypothesis to that of Blackwell and Gilbertson (1980) who observed that the short (5 d) life cycle on agar in *D. eremophilum* could be an adaptation to a fast-drying desert environment.

Blackwell and Gilbertson (1980) thought the small phaneroplasmodia of *Didymium eremophilum* was an adaptation to the extreme dry conditions of its environment and an example of evolutionary reduction. *Didymium operculatum* and the three other aforementioned species also have small phaneroplasmodia, producing a few sporocarps each and giving rise to their dispersed habit. This might be due to culture conditions, but it would be advantageous to have several small plasmodia instead of one large one to maximize resources and species survival by spreading into different parts of a rapidly drying environment. In moist chamber culture some larger plasmodia were seen to have nonsynchronous fruiting, with parts of the same plasmodium forming sporocarps over several days. This also has been observed in another myxomycete species, *D. wildpretii*, from arid areas (Lado et al. 2007b).

Ecology.—As pointed out by Wrigley de Basanta et al. (2009) genus *Didymium* is particularly abundant in surveys of arid regions (Novozhilov et al. 2006, Lado et al. 2007a, Lado et al. in press, Estrada-Torres et al. 2009). The *Didymium* species described from arid environments in the past few decades include *D. eremophilum*, *D. infundibuliforme*, *D. mexicanum* G. Moreno, Lizárraga & Illana, *D. reticulosporum*, *D. subreticulosporum*, *D. tehuacanense* Estrada, D. Wrigley & Lado, *D. umbilicatum* and *D. wildpretii* Mosquera, Estrada, Beltrán-Tej., D. Wrigley & Lado (TABLE I), but the species of the genus, commonly found in or associated with xeric conditions, number more than *25*.

Some adaptive characters, as discussed above, enabling this genus to colonize such extreme environments seem to be production of many small phaneroplasmodia, rapid and repeated microcyst/sclerotium formation resulting in the option of a longer life cycle. It appears that this genus of myxomycete particularly has evolved and diversified rapidly to exploit the slightly different microhabitat conditions in the plants that are able to grow in these arid environments. This might be an example of an adaptive radiation of the genus to occupy specific niches in particular plant species in desert areas and suggests co-evolution with the specific plants or plant groups that are their substrates. It is certainly becoming evident that in myxomycetes there is some species-substrate specialization and that increasing the study of different plant species produces closely related myxomycete species but with significant morphological differences. An example is this new *Didymium operculatum*, found on *Copiapoa* spp., an endemic genus of cactus in Chile (FIG. 22) but not found on the more than 30 other species of cacti examined or the several hundred moist chamber cultures of cacti completed by the authors in arid

FIG. 22. *Copiapoa* sp. the substrate for *Didymium operculatum*, Atacama, Chile.

environments in different parts of the two American continents. A similar situation was discussed for another myxomycete, *Licea eremophila* D. Wrigley, Lado & Estrada (Wrigley de Basanta et al. 2010), from arid areas found on species of the bromeliad genus *Puya* spp. in South America but not on the similar bromeliad genus *Hechtia* in Mexico. These examples in addition to our results for other newly described species (Estrada-Torres et al. 2009; Lado et al. 2009; Wrigley de Basanta et al. 2008, 2009), all of which are exclusive to groups of plants or even to one plant genus such as *Didymium operculatum* (see TABLE I) or species give further evidence to refute the ubiquity concept (Fenchel and Finlay 2004) for microorganism dispersal patterns and support the fact that the patterns of myxomycete distribution are more consistent with a moderate endemicity model, similar to that known for higher animals and plants (Foissner 2006), as suggested by Stephenson et al. (2008). DNA analyses to trace the evolutionary relationships of this ecological group should give new insight into the history of their relationships, but in any event the presence of these specific microorganisms in endemic plants suggests another urgent reason for conservation of plant biodiversity, and with it its microbial diversity.

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