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Description and culture of a new succulenticolous *Didymium* (Myxomycetes)

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Abstract: A new succulenticolous Myxomycete species, *Didymium wildpretii*, found on decaying remains of various species of cacti, is described from two arid zones of the world. This species was collected from central Mexico, at the southern limit of the Chihuahuan Desert, and from the Canary Islands (Spain). The new species has small, pale yellow sporocarps, 0.1–0.7 mm high, that are sessile or have short, orange-yellow, calcareous stalks and small, uniformly warted spores. The stability of the taxonomic characters of the species was confirmed with both moist chamber cultures and spore-to-spore culture on agar. Life cycle events are described from germination to sporulation. Myxomycete specimens were examined with scanning electron microscopy and light microscopy, and micrographs of relevant morphological details are included.

Key words: agar culture, arid zones, life cycle, moist chamber culture, morphogenesis, taxonomy

INTRODUCTION

The arid regions of the world are particularly fragile, and biodiversity in these areas is under threat from climate change and human development. The study and protection of these areas has been declared a priority of several international conventions (IPCC 1995, 2001). Myxomycete diversity of the arid areas of Mexico and the Canary Islands, Spain, has been the subject of intensive research over the past decade. These two biogeographical regions (neotropics and Maraconesia), separated by more than 6000 km, have some similar climatic factors and vegetation dominated by cacti and other succulents. The research has resulted in the description of several new Myxomycete species that seem to be particularly adapted to grow on decaying cacti (Lado et al 1999, Mosquera et al 2000, 2003, Estrada-Torres et al 2001). These and other succulenticolous species described from xeric environments are listed (TABLE I) and indicate that these fragile areas are particularly rich in Myxomycetes.

In this paper we describe a new *Didymium* that was obtained directly in the field in Mexico and from moist chamber cultures of substrates from Mexico and the Canary Islands (Spain). Because of the variability of some *Didymium* species (Clark and Mires 1999, ElHage et al 2000, Clark and Landolt 2001), this species also was cultured from spore-to-spore on agar to verify the stability of its taxonomic characters.

MATERIALS AND METHODS

The study is based on material obtained in the field in Mexico (Guanajuato State), moist chamber cultures of substrates collected in Tenerife (Canary Islands, Spain), Morelos and Puebla (Mexico) and also that obtained from spore-to-spore cultures on agar. All localities have been georeferenced by GPS. All specimens are deposited in the herbaria MA-Fungi (sub Lado), TFC Mic. and TLXM (sub E. Conde & A. Estrada-Torres). The collections on TLXM are abbreviated in this paper as EC-AET.

Moist chamber cultures.—The method used to set up moist chamber cultures of plant remains from arid areas was that described by Lado et al (1999). To evaluate the relative abundance of Myxomycete species on substrates in Mexico, 25 moist chambers of decaying remains of each the following 10 cacti were prepared. The substrates, all of which are relatively common in the Tehuacan Valley, were *Cephalocereus columna-trajani*, *Echinocactus platyacanthus*, *Ferocatus latispinus*, *Mammilaria carnea*, *Myrtillocactus geometrizans*, *Opuntia depressa*, *O. pilifera*, *O. tomentosa*, *Pachycereus hollianus* and *P. weberi*.

Agar cultures.—Isolates (dwb-1 and dwb-2) used for spore-to-spore culture were from mature sporocarps obtained from decaying remains of cacti in moist chamber culture (collections *EC-AET* 424 and 420, respectively). The spores were sown on 0.75% water agar (WA) at pH 7.0. The sporocarps were crushed and lightly applied to the agar in four quadrants of sterile 9 cm plastic Petri dishes. Cultures were kept at room temperature (21–23 C) with an approximate 12 h light-dark regime. Details of media and techniques can be found in Haskins and Wrigley de Basanta (2007).

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TABLE I. Myxomycete species described from cacti and other succulent substrates

Species	Substrates	References	Locality of the Type
Badhamia grandispora Illana & G. Moreno	Dead cladodes of *Opuntia ficus-indica*	Illana et al (1992)	Spain (Cádiz)
Badhamia melanospora Speg.	On *Cereus peruvianus*	Spegazzini (1880)	Argentina
Cribraria fragilis Lado & Estrada	Dead tissue of *Stenocereus* sp.	Estrada-Torres et al (2001)	Mexico (Morelos)
Cribraria zonatispora Lado, Mosquera & Beltrán-Tej.	On inner woody tissue of decayed *Opuntia* spp. cladodia	Lado et al (1999)	Spain (Canary Islands), Mexico (Hidalgo)
Didymium eremophilum Blackwell & Gilbertson	Dead tissues of *Carnegiea gigantea*	Blackwell and Gilbertson (1980)	USA (Arizona)
Didymium mexicanum G. Moreno, Lizárraga & Illana	Dead *Agave schawii* and *Yucca* sp.	Lizárraga et al (1996)	Mexico (Baja California)
Didymium subreticulosporum Oltra, G. Moreno & Illana	Cladodes fallen of *Opuntia maxima*	Moreno et al (1996)	Spain (Valencia)
Licea suculenticola Mosquera, Lado, Estrada-Torres & Beltrán-Tej.	Decaying leaves of *Agave* spp., *Aeonium* sp., decaying remains of *Austrocylindropuntia exaltata*, *Euphorbia canariensis*, *Myrtillocactus geometrizans*, *Opuntia* spp., *Stenoreceus* sp., and bark of *Yucca filifera Opuntia streptacantha, and Nolina parviflora*	Mosquera et al (2003)	Spain (Canary Islands), Mexico (Hidalgo, Morelos, Puebla, Tlaxcala), USA (New Jersey)
Physarum spectabile Nann.-Bremek., Lado & G. Moreno	Cladodes of *Opuntia maxima*	Nannenga-Bremekamp et al (1984)	Spain (Canary Islands)
Trichia agaves (G. Moreno, Lizárraga & Illana) Mosquera, Lado, Estrada & Beltrán-Tej. (= *T. perichaenoides* Mosquera, Lado, Estrada & Beltrán-Tej.)	Decaying *Agave, Yucca, Hechtia* and *Opuntia*	Moreno et al (2000), Mosquera et al (2000)	Mexico (Baja California, Hidalgo, Oaxaca, Puebla, Tlaxcala, Veracruz), Spain (Almería, Valencia, Canary, Balearic islands)

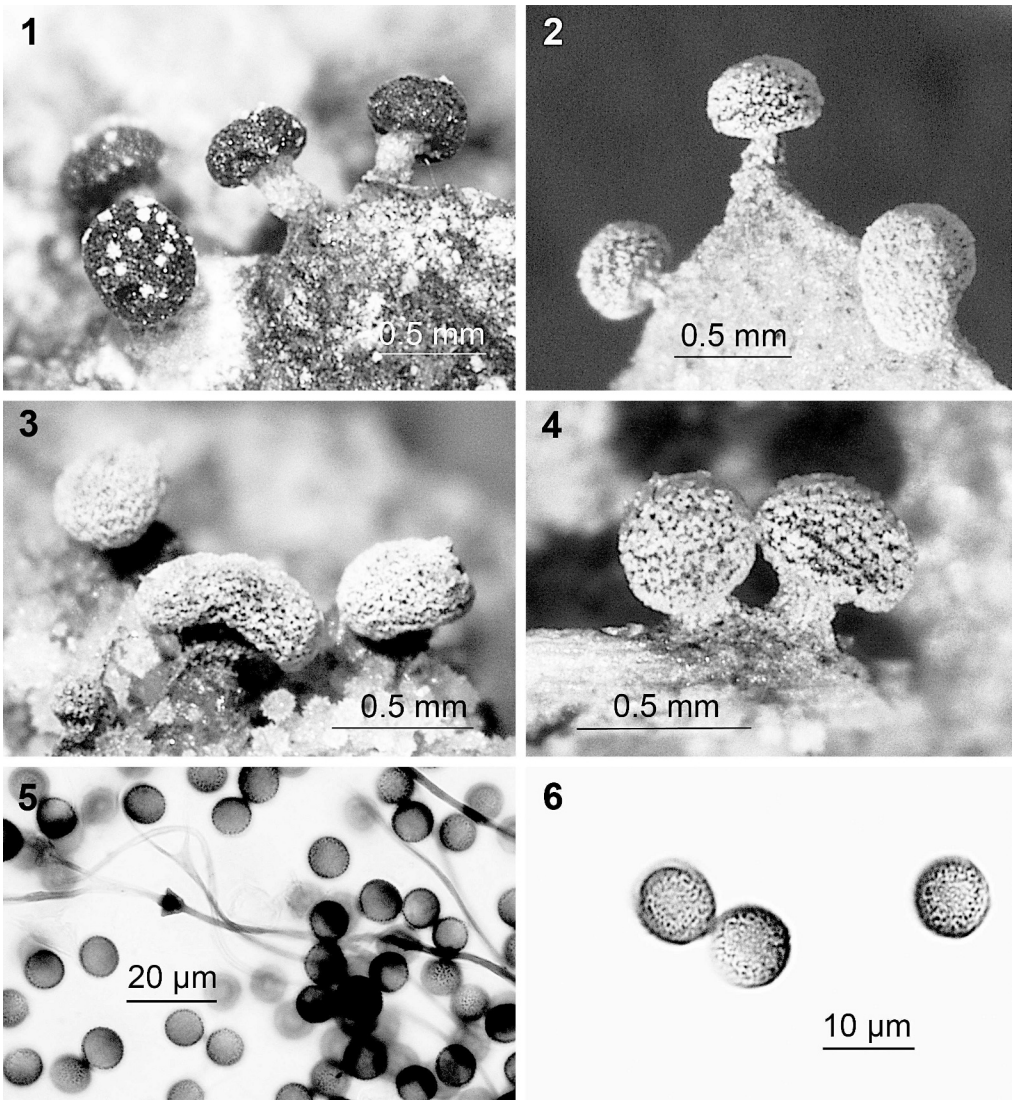
Microscopy.—Microscope measurements and observations were made with material mounted directly in Hoyer's medium. A microscopy with differential interference contrast (DIC) was used to obtain descriptive data and light micrographs. Critical-point drying was used for scanning electron microscopy (SEM) preparations, and the SEM analyses and photomicrographs of specimens were made by the scanning electron microscopy unit of the Royal Botanic Garden of Madrid, employing a Jeol T330A 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 wildpretii Mosquera, Estrada, Beltrán-Tej., D. Wrigley et Lado, sp. nov. FIGS. 1–26

Sporophora e sporocarpicis usque ad plasmodiocarpico, cum parvis stipitibus 0.1–0.7 mm in toto altitudine. Stipite calcareo, 0.1–0.3 mm alto, 0.1–0.3 mm lato. Sporotheca subhemisphaerica, flavescente vel albida. Peridio simplici, membranaceo, iridiscente, parcis calcis crystallis tecto. Sporis 7.5–9.5 μm diam, pardo-griseis cum parvo poro pallido germinante usque ad unum polum. Plasmodio pallido aurantiaco vel rufo.

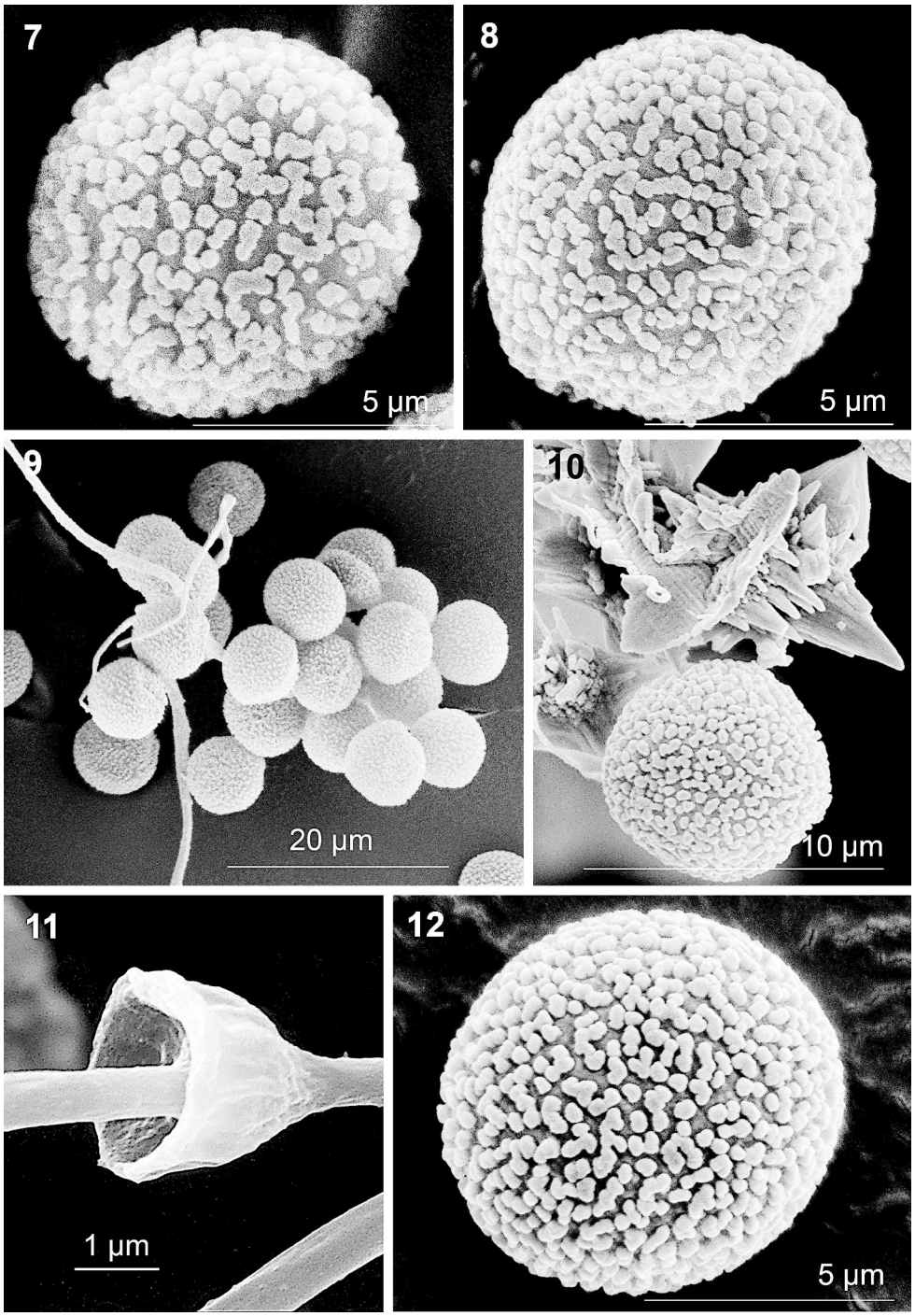
Sporophores usually sporocarpic, occasionally subplasmodiocarpic to plasmodiocarpic, dispersed or grouped. Sporocarps short-stalked to sessile, 0.1–0.7 mm high. Subplasmodiocarps and plasmodiocarps 1–2.5 mm long, reniform, occasionally effuse, sessile to short-stalked with multiple stalks. Sporotheca subhemisphaeric to slightly reniform, sometimes flattened below and then slightly umbilicate, 0.1–0.7 mm diam, pale orange-yellow (73. p. OY), light yellow (86. l. Y), pale yellow (89. p. Y) to whitish when almost covered with calcium carbonate crystals,



FIGS. 1–6. *Didymium wildpretii*. 1. Sporocarps (*EC-AET* 424). 2–4. Sporocarps (*EC-AET* 420). 5–6. Capillitum and spores (*TFC Mic. 8564*).

blackish and iridescent when the crystals are sparse or absent. Hypothallus insconspicuous, membranaceous, individualized to each sporophore and discoid, brownish orange, sometimes covered with calcium carbonate crystals and then almost white. Stalk short, cylindrical, sometimes broader at the apex, 0.1–0.3 mm high, 0.1–0.3 mm wide, filled with lime crystals and refuse matter, sometimes slightly striated, pale orange-yellow (73 p. OY) to strong orange-yellow (68. s. OY); deep orange-yellow (69. deep OY) by LM. Peridium single, iridescent, membranous, covered by pale yellow (89. p. Y), stellate lime crystals (FIG. 1); crystals forming an almost continuous roughened layer (FIGS. 2–4), sometimes scattered showing the iridescent peridium; dehiscence irregular. Columella absent or reduced, up to 0.2 mm high, limy, concolorous with the stalk. Capillitium arising radially from the columella, rigid, filiform (FIG. 5), threads 1–1.5 µm diam, scarcely branched, dark yellowish brown (78. d. y Br), turning pale to colorless through the ends, sometimes with vesicular enlargements and bell-shaped structures as seen by LM and SEM (FIGS. 5, 11). Spores free, brown black (65. br. Black) in mass, light brown (57. l. Br) to grayish brown (61 gy. Br) by LM, subglobose, (7.2−)7.5–9.5(−10.1) µm diam ($\tilde{x} \pm$ s.d. = 8.5 ± 0.65), densely and uniformely warted (FIGS. 6, 26), with some warts fused in a subreticulate pattern by SEM (FIGS. 7–10, 12). Phaneroplasmodium moderate orange-yellow (71. m. OY) to reddish orange (35. s. r O).

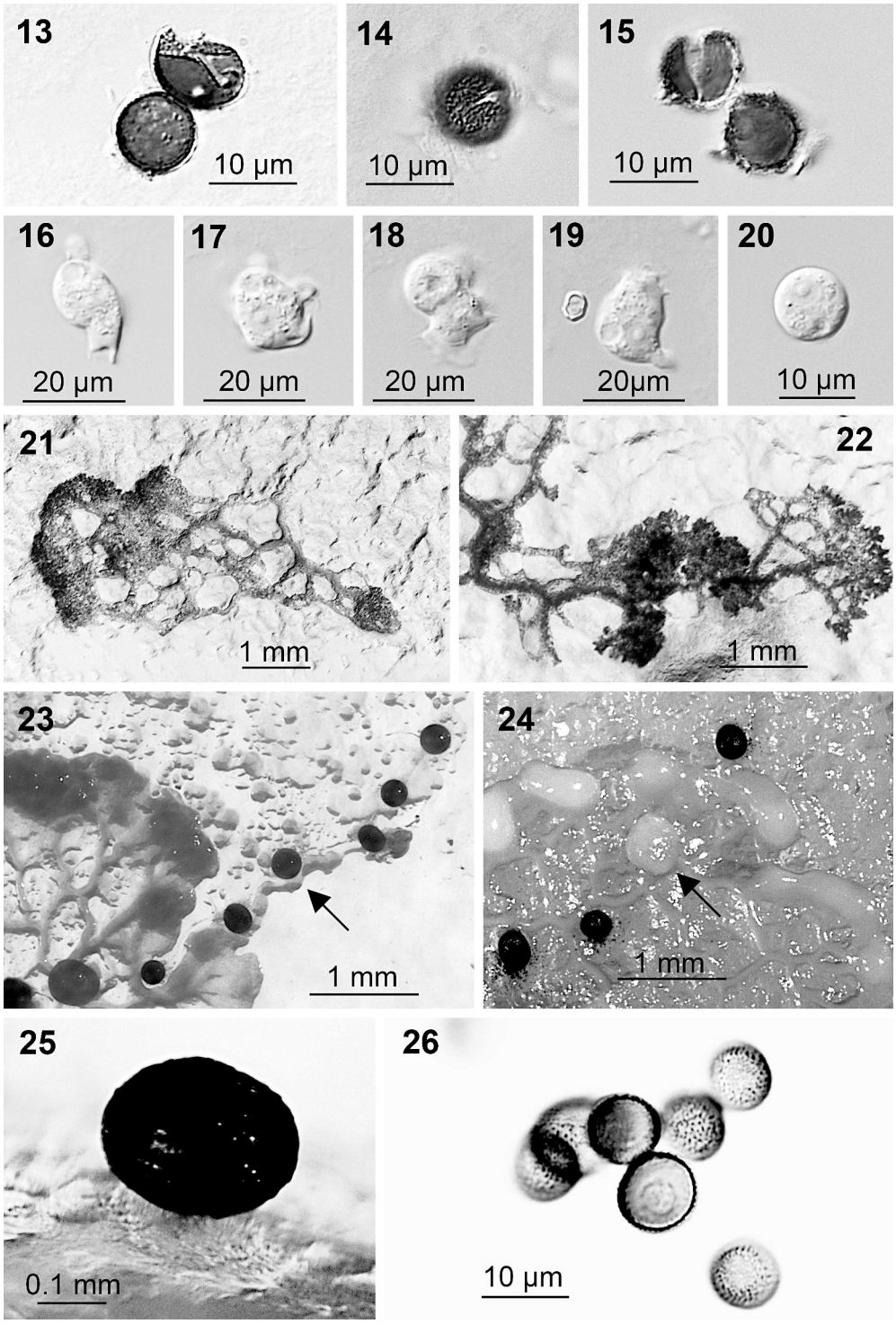
HOLOTYPE. MEXICO. Guanajuato, municipality of San Luis de la Paz, San Ernesto, 21°08′42″N,



FIGS. 7–12. *Didymium wildpretii* (SEM). 7. Spore (*EC-AET* 424). 8. Spore (*EC-AET* 420). 9. Spores and capillitium (*EC-AET* 422). 10. Spore and crystals of the peridium (*EC-AET* 422). 11. Capillitial thread showing a bell-shaped structure (*9138 Lado*). 12. Spore (EC-AET 422).

100°33′24″W, 2033 m, on decaying *Opuntia* sp., 22-IX-2002, *A. Estrada-Torres & C. Lado*, 13819 Lado (MA-Fungi 61104). Isotype TLXM (ET 8404).

Specimens examined. MEXICO. Morelos, Municipality of Tlaquiltenango, Sierra de Huautla Reserve, 18°27′25″N, 99°01′47″W, 990 m, on decaying remains of *Stenocereus* sp. in moist chamber culture, 12-V-1998, 9138 Lado. Puebla, Tehuacan-Cuicatlan Biosphere Reserve, Municipality of San Juan Miahuatlan, km 47 roadway Cuacnopalan-Oaxaca (Mex-135), 18°25′55″N, 97°25′07″W, 1689 m, on decaying remains of *Opuntia tomentosa* in moist chamber culture, 26-VII-2002, EC-AET 208a (pH = 8.6). Tehuacan-Cuicatlan



FIGS. 13–26. *Didymium wildpretii*. Spore-to-spore agar culture of *EC-AET* 420. 13–15. Germinating spores. 16–19. Myxamoebae. 20. Myxamoeba rounding to encyst. 21–22. Phaneroplasmodia among tracks on agar. 23–24. Development of sporocarps (arrows) along the edge of plasmodial fan. 25. Mature sporocarps showing discoid hypothallus. 26. Spores from a new generation sporocarp.

Biosphere Reserve, San Gabriel Chilac, km 69 roadway Cuacnopalan-Oaxaca (Mex-135), 18°16′40″N, 97°19′40″W, 1230 m, on decaying remains of *Echinocatus platyacanthus* in moist chamber culture, 15-VIII-2002, EC-AET 445 (pH = 7.8); ibidem, on decaying remains of *Ferocactus latispinus* in moist chamber culture, 27-VIII-2002, EC-AET 444 (pH = 9.1); ibidem, on decaying remains of *Mammilaria carnea* in moist chamber culture, 16-X-2002, EC-AET 441 (pH = 8.4);

ibidem, on decaying remains of *Myrtillocactus geometrizans* in moist chamber culture, 20-IX-2002, EC-AET 492 (pH = 8.5); ibidem, on decaying remains of *Pachycereus hollianus* in moist chamber culture, 31-VII-2002, EC-AET 412 (pH = 9.2). Tehuacan-Cuicatlan Biosphere Reserve, km 70 roadway Cuacnopalan-Oaxaca (Mex-135), 18°16′06″N, 97°19′06″W, 1200 m, on decaying remains of *Myrtillocactus geometrizans* in moist chamber culture, 10-III-2001, AET 7523. Tehuacan-Cuicatlan Biosphere Reserve, km 74 Cuacnopalan-Oaxaca roadway (Mex-135), 18°14′04″N, 97°17′05″W, 1200 m, on decaying remains of *Ferocactus latispinus* in moist chamber culture, 21-IV-2001, M.A. Flores & AET 139; ibidem, 2-VIII-2002, EC-AET 424 (pH = 9.0); on decaying remains of *Mammilaria carnea* in moist chamber culture, 31-VII-2002, EC-AET 419 (pH = 8.7); on decaying remains of *Opuntia depressa* in moist chamber culture, 27-VIII-2002, EC-AET 462 (pH = 8.4); on decaying remains of *Opuntia pilifera* in moist chamber culture, 31-VII-2002, EC-AET 327b (pH = 8.5), 14-VIII-2002, EC-AET 420 (pH = 9.2); ibidem, on decaying remains of *Opuntia* sp. in moist chamber culture, 31-III-2001, M.A. Flores & AET 42; on decaying remains of *Myrtillocactus geometrizans* in moist chamber culture, 6-IX-2002, EC-AET 422 (pH = 9.0); ibidem, on decaying remains of *Neobuxbaumia* sp. in moist chamber culture, 17-IV-2001, M.A. Flores & AET 112; ibidem, on decaying remains of *Pachycereus hollianus* in moist chamber culture, 13-VIII-2002, EC-AET 415 (pH = 9.2); on decaying remains of *Pachycereus weberii* in moist chamber culture, 4-IX-2002, EC-AET 414 (pH = 8.6). SPAIN. CANARY ISLANDS: Tenerife, Las Chafiras, San Miguel de Abona, 28°03′39″N, 16°36′57″W, 170 m, on decaying remains of *Opuntia* sp. in moist chamber culture, 2-II-1998, TFC Mic. 8270. Vilaflor, Jama, 28°07′26″N, 16°38′50″W, 1000 m, on decaying remains of *Opuntia maxima* in moist chamber culture, 24-III-1998, TFC Mic. 8537, 8564, 8567. San Juan de la Rambla, San José, 28°23′09″N, 16°39′04″W, 475 m, on decaying remains of *Opuntia maxima* in moist chamber culture, 1-III-1998, TFC Mic. 8534. El Boquerón, Valle de Guerra, 28°30′11″N, 16°23′16″W, 525 m, on decaying remains of *Opuntia maxima* in moist chamber culture, 24-III-1998, TFC Mic. 8572, 8673, 8674.

Etymology. Named after the botanist Dr Wolfredo Wildpret de la Torre, an expert in the flora of the Canary Islands.

Habitat. On decaying remains of several species of globose (*Echinocactus platyacanthus*, *Mammillaria carnea* and *Ferocactus latispinus*), opuntioid (*Opuntia depressa*, *O. maxima*, *O. pilifera* and *O. tomentosa*) and columnar (*Myrtillocactus geometrizans*, *Neobuxbaumia* sp., *Pachycereus hollianus*, *P. weberi* and *Stenocereus* sp.) cacti.

Distribution. Known from central Mexico (states of Guanajuato, Morelos and Puebla) and the Canary Islands. Possibly occurring in other regions of the world where cacti are present.

Moist chamber culture. Didymium wildpretii appeared in 78 of the 250 moist chamber cultures. The number of sporocarps developing in each moist chamber was variable, from only one to more than 300. The first fruiting bodies appeared 7 d after the moist chambers were set up, but in most cultures (66%) fructifications appeared at 21–42 d. In 74% of the cultures sporocarps were collected more than once and even up to 10 times from one culture. This extended the fruiting period from a few days up to 90 d maximum. The species appeared on different substrates with variable frequency. It was absent on *Cephalocereus columna-trajani*, appeared in two cultures of *Opuntia depressa*, three of *Echinocactus platyacanthus*, four cultures of *Ferocactus latispinus*, five of *Mammilaria carnea* and eight of *O. tomentosa*. Ten cultures of *O. pilifera* produced the new Myxomycete, 14 cultures of *Myrtillocactus geometrizans* and 16 cultures each of *Pachycereus hollianus* and *P. weberi*.

Agar culture.—Germination was by a v-shaped split in the spore wall (FIGS. 13–15). Forty eight hours after sowing the spores on agar in isolate dwb-2, all four quadrants had swarm cells 8–10 μm long and myxamoebae (FIGS. 16–19) which were dividing actively. Spores from only one quadrant of isolate dwb-1 germinated, and the isolate resulted only in myxamoebae. In isolate dwb-2 after 14 d some myxamoebae began to retract pseudopodia and differentiate into microcysts (FIG. 20), which signals adverse conditions according to Haskins at al (1985), in this case probably a diminished food supply. Small agar blocks, containing myxamoebae and swarm cells, were transferred from the germination plate to four fresh plates. The first plate had 1.5% water agar (WA) with only bacterial melange isolated incidentally with the spores, the second plate had 1.5% WA with streaks of *Escherichia coli*. The third plate had 1.5% WA with streaks of *E. coli* and sterile Quaker oat flour and half strength cornmeal agar (CM/2) with sterile Quaker oat flour was in the fourth plate. In the second plate the myxamoebal population grew rapidly forming small plasmodia in 5 d (FIGS. 21–22). The myxamoebae on agar with just bacterial melange did not form plasmodia, but after a week *E. coli* were added to the culture and 4 d later several reticulate plasmodia formed. Plasmodia also formed 5 d after the transfer of myxamoebae to the third and fourth plates. The plasmodia began as amoeboid, multinucleate, hyaline, young individuals looking like protoplasmodia, which showed minimal discontinuous cytoplasmic flow, every 20–40 s at 25 C, almost like a shudder. These grew to small phaneroplasmodia with a distinct leading edge, a reticulate zone behind (FIG. 21) and full reversible streaming. Color developed when they became phaneroplasmodia and was pale yellow at first

and darkened to a reddish orange as the plasmodia grew. Mature plasmodia spread out thinly when moving in abundant bacterial films and contained multiple food vacuoles, appearing almost lace-like (FIG. 22) with reduced cyclosis. Sporocarps began forming after a further 9 d (28 d total) with the spores darkening almost at once. Some small plasmodia produced single sporocarps, and larger plasmodia produced sporocarps all along the leading edge of the fan (FIGS. 23–24). Onset of sporulation was possibly the result of a sudden rise in ambient temperature to 27 C, which seemed to slow the cyclosis of all plasmodia. As the temperature was restored to normal, the rate of cyclosis increased. Sporulation continued over a period of more than 20 d, and sporocarps were produced from all plasmodia in all four plates. They were 0.3–0.5 mm diam for the sporothecae of stipitate forms, and with eight subplasmodiocarps to plasmodiocarps up to 2.5 mm long. There were also 13 sessile sporocarps from a total of more than 200 sporocarps that were formed from isolate dwb-2. The sporocarps were dried slowly and mounted in herbarium boxes, but most did not form lime crystals on the peridium.

DISCUSSION

The most distinctive characters of *Didymium wildpretii* are the size, only 0.1–0.7 mm high, the pale yellow sporotheca, and the short, robust, calcareous, orange-yellow stalk. The combination of these characters makes this species unique in the genus. *Didymium wildpretii* has a similar stalk to *D. vaccinum* (Durieu et Mont.) Buchet, but the lime of the outer peridium is roughened and not the shell-like layer typical of *D. vaccinum*. Its sporocarps are smaller (0.1–0.7 vs. 0.6–1 mm diam respectively) and its spores are uniformly and minutely warted, not sparsely marked with large warts as seen by LM in *D. vaccinum* (Martin and Alexopoulos 1969). The differences in spore ornamentation can be seen clearly by SEM. *Didymium vaccinum* has large dispersed warts to short, low crests shown by Lizárraga et al (1999, FIGS. 72–73), and *D. wildpretii* spores have warts that are dense, flat and uniform (FIGS. 7–10, 12), with some warts fused in a subreticulate pattern.

Other species that are almost sessile or have a short calcareous stalk are *D. obducens* P. Karst., the variable *D. squamulosum* (Alb. et Schwein.) Fr. and *D. mexicanum* G. Moreno, Lizárraga et Illana. The first can be differentiated by having larger spores (10–13 μm vs. 7.5–9.5 μm diam) with warts partially in rows forming ridges in some areas, almost smooth at the rest (Härkönen 1979, p 4), and the fruiting bodies are large, confluent sporocarps to effuse plasmodiocarps and not primarily individual sporocarps. *Didymium squamulosum* generally has larger and stalked white sporophores (< 1.5 mm vs. 0.1–0.7 mm high), the columella is prominent, white, hemispherical to discoid, and minute or absent in *D. wildpretii*; the spores by SEM have pilate ornamentation, and lines can be seen on the surface making it look polyhedral (Lizárraga et al 1999, FIGS. 70–71). *Didymium mexicanum*, another species recently described from Mexico from similar ecosystems, has much larger spores (14–16 μm diam vs. 7.5–9.5 μm diam in *D. wildpretii*), which are also polygonal in optical section, with strong ridges bearing warts united laterally in a reticulum visible by transmitted light (Moreno et al 1997, p 328) and subglobose and uniformly warted by transmitted light in *D. wildpretii*. By SEM the spores of *D. mexicanum* are different (Moreno et al 1997, FIGS. 10–13).

The abundant material from moist chambers confirms the characters seen in material obtained directly in the field. Material from agar culture had identical sporocarps and spores to the moist chamber collection from which it was isolated (FIGS. 25–26). The capillitium was also the same; however the sporocarps did not develop much lime.

Didymium wildpretii was described after studying more than 100 collections, most of them obtained from moist chamber cultures, because its size makes it easy to overlook in the field. From these results it seems that *Didymium wildpretii* is a relatively common species. It was obtained in approximately 30% of the moist chamber cultures studied, developing on almost all species of cactus used as substrates, except *Cephalocereus columna-trajani*. The abundance however was variable, with less than 10% of the cultures of *Echinocactus platyacanthus*, *Ferocactus latispinus* and *Opuntia depressa* positive for the new Myxomycete species but up to 50% of the *Myrtillocactus geometrizans* and *Pachycereus spp.* cultures producing it. This suggests a marked preference for columnar cacti. A total of 67.2% of the collections cited were on columnar cacti (*Myrtillocactus geometrizans*, *Pachycereus hollianus* and *P. weberi*), 21.5% on opuntioid cacti (*Opuntia depressa*, *O. pilifera* and *O. tomentosa*) and only 11.3% on globose cacti (*Echinocactus platyacanthus*, *Ferocactus latispinus* and *Mammilaria carnea*). In some cases the columnar cacti produced large fruiting of more than 75 sporocarps, whereas the globose cacti cultures produced fewer than 15 dispersed sporocarps. The species also has been obtained in other moist chamber cultures of *Neobuxbaumia* sp., *Opuntia ficus-indica* and *Stenocereus* sp. and collected in the field on decaying *Opuntia* sp. With the data obtained up to now *D. wildpretii* certainly developed on the decaying remains of

diverse species of cacti, especially on the sclerified parenchyma and woody vascular tissue of the inside of the plant. It remains to be seen whether it also can develop on other succulents.

Didymium wildpretii grows on substrates with basic pH values, 7.8–10.0, with the optimum at 8.5–9.4, values in which 76% of the fructifications were obtained in moist chamber cultures. This pH does not appear to be a critical condition for agar culture because the media were all circum-neutral and numerous fruiting bodies were produced. The reason for the lack of lime deposits on the peridium of the material from agar culture however could be the neutral pH of the medium. This phenomenon has been observed in fructifications formed on agar in *Didymium annulisporum* H.W. Keller & Schokn. (Keller and Schoknecht 1989a) and *Badhamia rhytidosperma* H.W. Keller & Schokn. (Keller and Schoknecht 1989b). Perhaps the basic pH of the natural medium (decaying remains of cacti) encourages calcium storage in the plasmodia and permits greater calcification of the peridium. Another possibility is that the lack of lime deposit is due to the excess moisture from the agar.

Germination in culture can vary from a few hours to several days even in the same species, depending on the technique used for germination (Gray and Alexopoulos 1968), and some spores can take up to a month (Haskins and Wrigley de Basanta 2007). *Didymium wildpretii* took under 48 h to produce large numbers of myxomonads, over twice the time taken by another *Didymium* species reported in the literature, *D. annulisporum*, which took about 19 h to germinate (Keller and Schoknecht 1989a). After germination of *D. wildpretii* spores a mixture of swarm cells and myxamoebae was observed on the aqueous 0.75% WA, but on the drier 1.5% WA most of the cells reverted to myxamoebae. The myxamoebae had one or two contractile vacuoles, which still were visible when they were rounding to form microcysts (FIG. 20) but not visible in the microcysts themselves once formed. This agrees with observations reported by Haskins et al (1985) in *Echinostelium minutum*. Microcysts excysted when agar blocks were transferred to the new media with food.

The type and quantity of food seems to be critical at certain stages of plasmodial development. When sterile Quaker oat flour was added to one of the cultures, even after sporulation had begun for some plasmodia, it encouraged significant growth in other plasmodia present, over a period of several days. The oats seem to stimulate plasmodial growth. These results agree with those of Keller and Schoknecht (1989a) because in *Didymium annulisporum* larger plasmodia were reported when Quaker oats were added to agar cultures with *E. coli* and smaller plasmodia were produced with *E. coli* alone. Keller and Schoknecht (1989a) suggested that this might be because the oats either add nutrients for the bacteria or for the plasmodium directly. In *D. wildpretii* these larger plasmodia were much darker.

The life cycle on agar of *Didymium wildpretii* was 28–56 d. Incubation from wetting the substrate to sporulation in moist chambers was as little as 7 d in some cases, but the majority took 21–42 d, similar to the life cycle on agar. The shorter cycles in some moist chamber cultures suggest that the Myxomycete was already in the form of sclerotia on the cacti when placed in culture. In general incubation in moist chamber culture of bark can vary from a few days to several months (Wrigley de Basanta 1998), but with these cultures it is never possible to know whether the bark had the initiate in the form of spores, microcysts or sclerotia. In *D. annulisporum* (Keller and Schoknecht 1989a) the life cycle on agar was completed in 6–9 d, much shorter than the month taken by *D. wildpretii*. In another genus in agar culture however a similar length life cycle has been recorded because *Perichaena depressa* and *P. quadrata* took 30–45 d (Keller and Eliasson 1992).

Didymium wildpretii has been found only inside cacti, both in the field and in moist chamber cultures. It is therefore a genuine succulenticolous species as defined by Lado et al (1999). It seems to be a generalist within this substrate group because it has been found on 13 species of cacti.

Although the habitat is always arid or semi-arid where this Myxomycete has been found, the geographic locations are distinct. In Mexico it was found in six localities of three states and on Tenerife in four localities. These cover a wide range of both latitude and longitude, 18–21°N in Mexico to 28°N in Tenerife, and from 97–100°W in Mexico to 16°W in Tenerife. The elevation also varied, 170–525 m in the Canary Islands to 990–2033 m in Mexico. However the *Opuntia* species, which were the sole substrates in the Canary Islands, are cactus species introduced from Mexico in the 16th century. It is interesting to note that *D. wildpretii* is at least the sixth case in which the substrate appears to have been accompanied by a Myxomycete. The other Myxomycetes found on cacti or succulents in both Mexico and Spain are *Licea succulenticola* (Mosquera et al 2003), *Trichia agaves* (G. Moreno, Lizárraga & Illana) Mosquera, Lado, Estrada & Beltrán-Tej. (Mosquera et al 2000), *Cribraria zonatispora* (Lado et al 1999), *Didymium subreticulosporum* Oltra, G. Moreno & Illana (Moreno et al 1996) and *Physarum spectabile* Nann.-Bremek., Lado & G. Moreno (Nannenga-Bremekamp et al 1984), described from the Canary Islands on *Opuntia*

and recently found abundantly by the authors in Mexico.

The size and dispersed habit of this Myxomycete, coupled with the fact that it can be mistaken for the crystalline substances formed in the decomposing substrate, mean that it probably has been overlooked. The material has been collected from distant localities, separated by more than 6000 km, in two biogeographical regions (neotropics and Macaronesia). In spite of this distribution the morphological characters were constant and the stability of its morphotaxonomic features has been confirmed by spore-to-spore culture. It therefore is described as a new species and adds to the growing number of succulenticolous Myxomycetes. The study of this species suggests that geographic factors do not directly affect the distribution of Myxomycetes. Instead it appears that distribution depends on specific abiotic factors of the microhabitat, in this case the interior of plants serving as substrate.

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