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Didymium xerophilum, a new myxomycete from the tropical Andes

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Abstract: A new species of *Didymium* (Myxomycetes), *D. xerophilum*, is described, and some details of its life cycle are provided. The new species was collected during studies of arid areas of Argentina and Peru. It can be distinguished by the persistent funnel-shaped invagination of the peridium, the top of which appears as a deep umbilicus in closed sporothecae, and the calcareous hypothallus shared among several sporocarps. This combination of characters, with a circumscissile dehiscence of the sporotheca and a cream stalk packed with rhombic lime crystals, is unknown in other described species. Morphology was examined with scanning electron microscopy and light microscopy, and micrographs of relevant details are included here. Phylogenetic analysis with 18S rDNA sequences of different species of *Didymium* supports the distinct identity of this new species. Some collections of this myxomycete were made at up to 4600 m, an altitude almost unknown for this group of microorganisms.

Key words: Amoebozoa, Neotropics, phylogeny, SEM, taxonomy, xerophiles

Introduction

The Myxotropic Project includes intense surveys of myxomycetes from arid areas in several countries of the Neotropics (Lado et al. 2007a, 2011, 2013; Estrada-Torres et al. 2009; Wrigley de Basanta et al. 2010, 2011, 2012). In some areas of Argentina and Peru, a myxomycete identified as a species of *Didymium*, which did not correspond to any described species, was collected several times.

Schrader (1797) originally described the myxomycete genus *Didymium* based on eight species. The species in this genus are distinguished from others by the crystalline nature of the peridial lime. *Didymium farinaceum* Shrad., now considered a synonym of *D. melanospermum* (Pers.) T. Macbr., is considered the type species and is the only one of the eight original species to remain classified in the genus (Martin and Alexopoulos 1969, Lado 2005–2014). Over the past few decades, many new species were added to the genus, which now includes more than 70 (Lado 2005–2014). The genus appears to be particularly well adapted to arid conditions; almost 21% of samples from a study of Atacama Desert in Chile and almost 20% from the Tehuacán-Cuicatlán Valley in Mexico belonged to this genus (Lado et al. 2007a, Estrada-Torres et al. 2009). *Didymium* spp. were among the recently described species from xeric environments (Wrigley de Basanta et al. 2008, 2009, 2011).

Material and methods

The study area was in the Andean puna that borders the Monte Desert in northern Argentina, in the steppe of the slopes of the Argentinean Andes and the puna of the Peruvian Andes, near the northern limit of the Atacama Desert. The area is 24–40S and 65–74W. The surveys were done yearly 2006–2013, and known or suspected habitats of myxomycetes were examined in the field. The areas where samples of this new myxomycete were collected are arid or semiarid with average annual temperature 3–24 C and mean annual rainfall less than 450 mm. Collecting was done in each country to coincide with the austral spring and the end of the summer of the same phenological year. During collection, there was no rainfall at any of the sites, although in Peru rainfall had occurred in the month before. Temperatures during collecting varied according to elevation, with temperatures close to 0 C at the highest elevations and up to 20 C at the lowest.

Collections were glued into herbarium boxes and dried in situ. All localities were geo-referenced with a GPS Garmin eTrex Vista HCX (datum WGS84). All specimens are deposited in the herbarium MA-Fungi (sub Lado), with some duplicates in TLXM and the private collection of the first author (dwb). Agar cultures were prepared with spores from mature sporocarps of collections Lado 22243 and 22247. Spores were sown on 0.75% water agar (WA) at pH 7.0. Sporocarps were crushed and spores released over the agar in each of four quadrants of sterile 9 cm plastic Petri dishes. The cultures were maintained in an incubator at 22 C, with an approximate 12 h light/dark regime. Details of media used and techniques followed can be found in Haskins and Wrigley de Basanta (2008). Sections of mature sporocarps were made to see details of the sporotheca and columella following procedures described by Matsumoto and Deguchi (1999). Material was mounted in Hoyer's medium for microscope measurements and

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observations. Measurements were made of 10 spores from each of the collections. Descriptive data and light micrographs were obtained with a Nikon Eclipse 80i microscope with differential interference contrast (DIC). Critical point drying was used for scanning electron microscopy (SEM) preparations and SEM analyses. Electron micrographs of specimens were made at the Scanning Electron Microscopy Department of the Royal Botanic Garden of Madrid, Spain, with a Hitachi S-3000N SEM at 10–15 kV. Color notations in parentheses are from the ISCC-NBS Color Name Charts Illustrated with Centroid Colors (Anon. 1976).

DNA extraction and 18S rDNA amplification.—Two specimens of the new species, including the type material, were selected for molecular studies. A further 30 samples belonging to other *Didymium* species, collected in widely distributed localities and deposited in the Myxomycete collection of the Royal Botanic Garden of Madrid (MA-Fungi, TABLE I), also were processed. DNA was extracted from 5–8 sporocarps, depending on abundance, with a DNeasy Plant Minikit (QIAGEN, United States). The samples were placed in 1.5 mL safe-lock Eppendorf tubes containing 25 µL AP1 buffer and incubated overnight at 65 C. Then they were shock-frozen with liquid nitrogen before being ground in an electric mortar approximately 5 min. To avoid melting while crushing, the samples were kept on ice. An additional 375 µL AP1 buffer was added and the remaining steps of the extraction procedure were performed according to the manufacturer's instructions. A fragment of the nuc small subunit rRNA gene (nuc 18S rDNA) of about 430 bp was amplified by PCR with a MyTaq™ Red Mix kit (Bioline, United Kingdom). Each reaction mixture contained 12.5 µL MyTaq™ Red Mix, 1 µL each primer, 3 µL DNA template (approx. 10 ng/µL) and Milli-Q water to a final volume of 25 µL. The primer pair used was Phf1b-A (AAA ACT CAC CAG GTC CAG AT)/JKr–2 (AGG GCA GGG ACG CAT TC). The direct primer was a modification from Kamono and Fukui (2006) and the reverse primer was designed in this study for *Didymium*. PCR amplification was carried out with these cycling parameters: an initial denaturation step at 94 C for 3 min, followed by 30 cycles of denaturation at 94 C for 60 s, annealing at 58 C for 60 s and polymerization at 72 C for 2 min, with a final extension step at 72 C for 10 min.

Sequencing, assembly and alignment.—Each PCR product was directly sequenced in forward and reverse directions with an ABI 3730XL automatic sequencer (Macrogen, Korea). The assembly of both strands and the subsequent manual edition of consensus sequences were performed in Geneious 5.0 (Drummond et al. 2010). The new nuclear 18S rDNA sequences were automatically aligned with default settings in MAFFT 7.017 (Katoh et al. 2002), with other *Didymium* GenBank sequences (TABLE II), based on BLAST queries (maximum identity > 92%). Four additional sequences (DQ903679, AB259447, HE614609, HE614617) representing other genera of the Didymiaceae (*Mucilago*, *Lepidoderma*, *Diderma*) also were selected. These three genera are closely related to *Didymium*, according to phylogenetic evidence (Fiore-Donno et al. 2008, Nandipati et al. 2012). They were included in the alignment for a better placement of the new species, not only within this genus but also within the Didymiaceae. Finally the data matrix also included two sequences of *Fuligo septica* (KJ545570, KJ545569) and one of *Physarum roseum* (HE614605) as the most external outgroup to root the trees. Both species of the family Physaraceae also are related to genus *Didymium* but more distantly (Nandipati et al. 2010).

Missing or ambiguous characters were coded as N and treated as missing data. The automatic alignment was visually examined in Geneious 5.0 so minor errors were corrected and endings of the longest sequences were trimmed, respectively. All sequences newly obtained in this study were submitted to GenBank, under accession numbers KJ545537–KJ545570 (TABLE I), and the final alignment was deposited in TreeBASE (http://www.treebase.org; TB2:15505).

Phylogenetic analysis.—GTR + Γ was selected as the best-fit model of nucleotide substitution under Akaike's information criterion (AIC) in jModelTest 2 (Darriba et al. 2012). Phylogenetic analyses were done with both maximum likelihood (ML) and Bayesian inference (BI). The ML analysis was performed in RAxMLGUI 1.3 (Stamatakis 2006, Silvestro and Michalak 2012) using the rapid bootstrap algorithm option and 1000 nonparametric bootstrap replicates for assessing branch support (BS). The BI analysis was executed in MrBayes 3.2 (Ronquist et al. 2012) and consisted of two independent runs of 10 000 000 generations, each one starting with a random tree and using four independent Monte Carlo Markov chains (MCMC). The convergence of both runs was assessed through the effective sampling size criterion for each parameter in Tracer 1.5 (Rambaut et al. 2013). Branch-length estimates of a preliminary Bayesian tree, built with default priors, were much longer than those corresponding to ML analysis. In fact, the intervals of the Bayesian branch-length estimates did not include those from ML analysis, which can be considered clear evidence of biased Bayesian estimates (Brown et al. 2010). To get accurate results, the default value of the parameter that controls branch lengths (λ = 10) was changed by λ = 50, with the command used by Zamora et al. (2014). After correcting this parameter, a new Bayesian analysis was conducted.

Trees were sampled every 1000 generations. The initial 25% of trees sampled was discarded as burn-in; the tree was calculated with the remainder. Posterior probabilities (PP) were obtained (Larget and Simon 1999, Huelsenbeck and Ronquist 2001) by sampling trees using the Markov chain Monte Carlo (MCMC) method. A combination of both bootstrap (BS) values and posterior probabilities (PP) was used to test the confidence of each branch according to the scale proposed by Lutzoni et al. (2004). Phylogenetic trees were visualized and edited with FigTree (Rambaut 2013), and artwork was processed with Adobe® Illustrator.

RESULTS

Phylogenetic analysis.—Thirty-two new nuclear 18S rDNA sequences of species of *Didymium* and two of

TABLE I. Data on MA-Fungi collections used for nuc 18S rDNA sequences

Herbarium No. MA-Fungi	Species	Country	Coordinates	Alt. (m)	Date	Collection No.	GenBank accession No.	Seq length (bp)
73335	*Didymium anellus*	Spain	39°08′08″N, 00°25′12″W	30	02-I-2005	Oltra 8420	KJ545537	392
69838	*D. bahiense*	Brazil	22°48′50″S, 48°44′35″W	720	23-XI-2004	Lado 17327	KJ545538	426
51663	*D. clavus*	Ecuador	00°40′24″S, 76°22′47″W	210	06-IV-2000	Lado 11766	KJ545539	422
81616	*D. clavus*	Madagascar	25°01′34″S, 46°59′15″E	17	15-V-2009	Lado 19891	KJ545540	422
80797	*D. crustaceum*	Chile	32°52′11″S, 70°50′55″W	628	07-IV-2006	Lado 17991	KJ545541	425
64529	*D. difforme*	Spain	43°08′15″N, 06°15′43″W	600	06-V-2005	Lado 17499	KJ545542	429
63904	*D. dubium*	Italy	44°15′35″N, 07°02′42″E	1650	11-V-2002	Lado 13756	KJ545543	427
80036	*D. dubium*	France	—	1450	21-IV-2003	Meyer 22726	KJ545544	427
46771	*D. floccosum*	Ecuador	00°07′01″N, 78°37′48″E	—	03-XII-1998	Lado 10033	KJ545545	397
64472	*D. floccosum*	Mexico	18°16′40″N, 97°19′39″W	1230	08-X-1999	Lado 10931	KJ545546	429
78324	*D. infundibuliforme*	Argentina	30°21′03″S, 68°38′07″W	1054	08-III-2007	Lado 18708	KJ545547	416
81548	*D. intermedium*	Madagascar	22°08′07″S, 46°53′27″E	1625	12-V-2009	Lado 19820	KJ545548	428
52082	*D. megalosporum*	Spain	40°43′04″N, 04°05′21″W	1076	20-II-2000	Oltra 3707	KJ545549	425
80635	*D. megalosporum*	Chile	33°09′19″S, 71°32′16″W	350	30-III-2006	Lado 17801	KJ545550	428
82049	*D. megalosporum*	Spain	39°22′04″N, 00°19′49″W	—	17-XII-2011	Oltra 12669	KJ545551	425
69847	*D. minus*	Brazil	22°48′50″S, 48°44′35″W	720	23-XI-2004	Lado 17335	KJ545552	428
80617	*D. nigripes*	Chile	37°47′41″S, 72°51′08″W	623	29-III-2006	Lado 17777	KJ545553	433
80820	*D. operculatum*	Chile	24°41′53″S, 70°33′43″W	36	18-II-2008	Lado 19083	KJ545554	394
74050	*D. operculatum* [a]	Chile	24°25′57″S, 70°31′59″W	137	28-II-2009	Dwb 3142	KJ545555	422
69000	*D. pertusum*	Spain	38°42′28″N, 00°37′51″W	740	26-V-2006	Oltra 9221	KJ545556	430
69001	*D. pertusum*	Spain	38°42′28″N, 00°37′51″W	740	26-V-2006	Oltra 9222	KJ545557	430
82075	*D. serpula*	Spain	38°41′57″N, 00°39′15″W	720	02-I-2012	Oltra 12699	KJ545558	334
78327	*Didymium* sp.	Chile	25°00′19″S, 70°24′23″W	935	21-I-2004	Lado 15611	KJ545559	429
80292	*D. squamulosum*	Argentina	28°59′00″S, 67°30′51″W	1390	29-XI-2006	Lado 18413	KJ545560	433
86903	*D. squamulosum*	Spain	40°20′24″N, 04°21′34″W	750	17-XI-2013	—	KJ545561	428
73605	*D. tehuacanense* [a]	Mexico	18°43′58″N, 97°31′47″W	2412	08-VII-2003	Lado 14784	KJ545562	396
64629	*D. umbilicatum*	Mexico	20°36′53″N, 98°59′12″W	1830	14-XI-1999	Lado 11206	KJ545563	428
64297	*D. umbilicatum* cf.	Mexico	19°31′31″N, 97°18′46″W	2380	02-X-1999	Lado 10653	KJ545564	420
81429	*D. verrucosporum*	Madagascar	21°15′31″S, 47°25′15″E	914	10-V-2009	Lado 19696	KJ545565	428
80992	*D. wildpretii*	Mexico	18°14′04″N, 97°17′05″W	—	21-X-2000	Conde-Cano 420	KJ545566	427
86877	*D. xerophilum*	Argentina	40°01′02″S, 70°49′24″W	668	05-XI-2009	Lado 20274	KJ545567	392
86885	*D. xerophilum* [a]	Peru	15°52′40″S, 71°08′54″W	4444	08-X-2012	Lado 22243	KJ545568	427
41447	*Fuligo septica*	Spain	40°34′39″N, 03°41′38″W	719	13-IX-1999	—	KJ545569	427
42182	*Fuligo septica*	Spain	40°23′08″N, 03°57′16″W	575	27-IV-1998	Oltra 2724	KJ545570	427

[a] Type material.

TABLE II. Data on GenBank sequences used for phylogenetic analysis

GenBank accession No.	Species	Trimmed seq. length (bp)	Voucher No.
HE614617	*Diderma niveum*	1240	Uk-K79
AB259387	*Didymium bahiense*	427	TNS-M-Y-4944
AB259388	*D. bahiense*	427	TNS-M-Y-4948
AB259389	*D. clavus*	422	JM-4507
AB259390	*D. clavus*	421	AK-04172
AB259391	*D. clavus*	422	AK-04296
AB259392	*D. clavus*	421	TNS-M-Y-17152
AB259395	*D. crustaceum*	424	TNS-M-Y-17612
AB259396	*D. crustaceum*	424	YY-26183
AM231294	*D. dubium*	427	K7
AM231295	*D. dubium*	427	K15
AB259399	*D. dubium*	426	TNS-M-Y-17046
AB435325	*D. dubium*	426	AK-06007
AB435326	*D. dubium*	426	AK-06010
AB435327	*D. dubium*	426	AK-06018
AB259402	*D. floccoides*	428	AK-04032
AB259403	*D. floccoides*	429	AK-04046
AB259404	*D. floccoides*	428	AK-04075
AB259405	*D. floccosum*	434	TNS-M-Y-16882
AB259406	*D. floccosum*	434	JM-3011
AB259407	*D. iridis*	429	JM-S-08
AB259408	*D. iridis*	433	JM-643
AB259409	*D. iridis*	425	AK-04205
AB259410	*D. laccatipes*	428	AK-F028
AB259413	*D. marineri*	426	TNS-M-Y-15365
AB259414	*D. megalosporum*	426	JM-S-06
AB259415	*D. megalosporum*	426	JM-4509
AB259416	*D. megalosporum*	426	AK-03035
AB259417	*D. megalosporum*	426	AK-04125
AB259418	*D. megalosporum*	426	AK-04156
AB259424	*D. nigripes*	429	JM-S-03
AB259425	*D. nigripes*	427	JM-S-09
AB259426	*D. nigripes*	433	JM-4513
AB259427	*D. nigripes*	429	AK-04154
AB435333	*D. nigripes*	433	AK-05137
AB435334	*D. nigripes*	425	AK-06080
AB435335	*D. nigripes*	433	AK-06100
AB435336	*D. nigripes*	433	AK-06110
AF239230	*D. nigripes*	426	—
AB435337	*D. squamulosum*	432	AK-06063
AB435338	*D. squamulosum*	434	AK-06085
AB435339	*D. squamulosum*	434	AK-06119
AB259430	*D. squamulosum*	426	JM-3620
AB259431	*D. squamulosum*	426	AK-03050
AB259432	*D. squamulosum*	434	AK-04009
AB259433	*D. squamulosum*	426	AK-02014
AB259434	*D. squamulosum*	434	AK-04111
AB259435	*D. squamulosum*	426	AK-04124
AB259436	*D. squamulosum*	434	AK-04126
AB259437	*D. squamulosum*	426	AK-04134
AB259438	*D. squamulosum*	430	AK-04413
AM231293	*D. squamulosum*	427	CR10
AY321111	*D.* sp.	428	E2/8
AY321112	*D.* sp.	429	E3P
EF118757	*D.* sp.	426	CH14/I
EF118758	*D.* sp.	426	CH1/I

TABLE II. Continued

GenBank accession No.	Species	Trimmed seq. length (bp)	Voucher No.
EF118759	*D.* sp.	426	CH54/I
EF118760	*D.* sp.	426	CH43/I
EF118761	*D.* sp.	426	CH49/I
HE614609	*Lepidoderma carestianum*	432	Fr-K18
AB259447	*Mucilago crustacea*	424	JM-4267
DQ903679	*Mucilago crustacea*	424	MM 24347
HE614605	*Physarum roseum*	420	C1

Fuligo were generated. Among them, two identical sequences from different specimens of the newly described species *D. xerophilum* were found (FIG. 1). The final data matrix used for phylogenetic analyses consisted of 97 sequences and 1256 characters. A total of 1075 sites were constant, 32 were uninformative and the remaining 149 variable characters were informative. After the modification of the parameter λ, the corrected BI analysis yielded more similar branch-length estimates to those from ML analysis than the preliminary analysis with default priors. Hereafter only the Bayesian tree resulting from the corrected analysis is discussed.

Overall there was topological congruence between ML and BI analyses, with just a few minor incongruences that were not well supported. The position of the new species *D. xerophilum* did not vary in the different approaches. The ML analysis yielded a tree with a larger number of resolved nodes but with very low statistical support, while BI produced a consensus tree with support values that in general were higher than those from ML analysis. Consequently only the Bayesian consensus tree, showing both BI posterior probabilities (PP) and ML bootstrap values (BS), is provided here (FIG. 1). Different analytical methods (i.e. ML, BI) produced highly consistent results, and reasonable support was obtained for the great majority of the clades nested within the highlighted group. Relationships among some clades, which are not very closely related to the group under study here, were strong enough to allow these parts of the tree to be collapsed, in the interest of brevity and clarity. These clades were given the name of one representative species (see *D. nigripes* group) and only the part of the tree comprising the new species is highlighted and discussed in more detail (FIG. 1).

Life cycle in agar culture.—Spores germinated by a V-shaped split in the spore wall typical of the genus (FIG. 2). The myxamoebae were large (FIG. 3) and actively engulfed the bacteria carried into the germination plate along with the spores. Spores from samples Lado 22243 and Lado 22247 germinated in 48–72 h. When microcysts were transferred from the germination plate to wMY agar (Lado 22243), they excysted to make swarm cells in only 10 min. The addition of sterile *Parastrephia lepidophylla* extract as a nutrient solution improved germination and the growth of amoebae, particularly on CM/2 agar. Amoebae grew vigorously and formed early plasmodia in several isolates (FIGS. 4, 5) but then encysted repeatedly (FIG. 6) and failed to form larger plasmodia, despite many trials with different media and nutrients. The reason for this failure is unknown, but perhaps factors from the natural environment necessary for the later stages of the life cycle are lacking in the closed cultures.

TAXONOMY

Didymium xerophilum Lado, Estrada et D. Wrigley, sp. nov. FIGS. 7–20

MycoBank MB808159

Holotype: PERU. Arequipa: Caylloma, San Antonio de Chuca, route PE-34A, km 157, 18 km west of Imata, 15°52′40″S, 71°08′54″W, 4444 m, on leaf litter and twigs of *Parastrephia phyliciformis*, 8 Oct 2012, *A. Estrada, C. Lado, I. Treviño & D. Wrigley de Basanta, Lado 22243* (MA-Fungi 86885).

Etymology: xerophilum (Gr.), loving dry places.

Sporophores sporocarpic, grouped, sometimes on a vein-like common hypothallus. Sporocarps 0.75–2 mm total height, stipitate (FIGS. 7–8). Sporotheca slightly oblate to subhemispheric, umbilicate above (FIGS. 7, 9–10), 0.5–2.0 mm diam, covered with lime crystals, light gray (264. l. Gray) to white (263. White), especially above, occasionally iridescent below, where the crystals are sparse or absent. Stalk erect, subcylindrical, expanded at the base (FIGS. 8–9, 11), 0.25–1.5 mm long, 0.15–0.25 mm diam, calcareous, packed inside with rhomboid lime crystals (FIG. 13) and with stellate crystals on the surface of the upper portion only (FIG. 15), ending as a columella inside the sporotheca (FIGS. 9, 11–12, 15), yellowish white (86. l. Y–89. p. Y) to olive-brown (94. l. Ol Br), occasionally yellowish brown (75. deep y Br–78. d. y Br) in reflected light, pale yellow (86. l. Y–84. s. Y) to orange-yellow (67. brill. OY–72. d. OY) by LM.

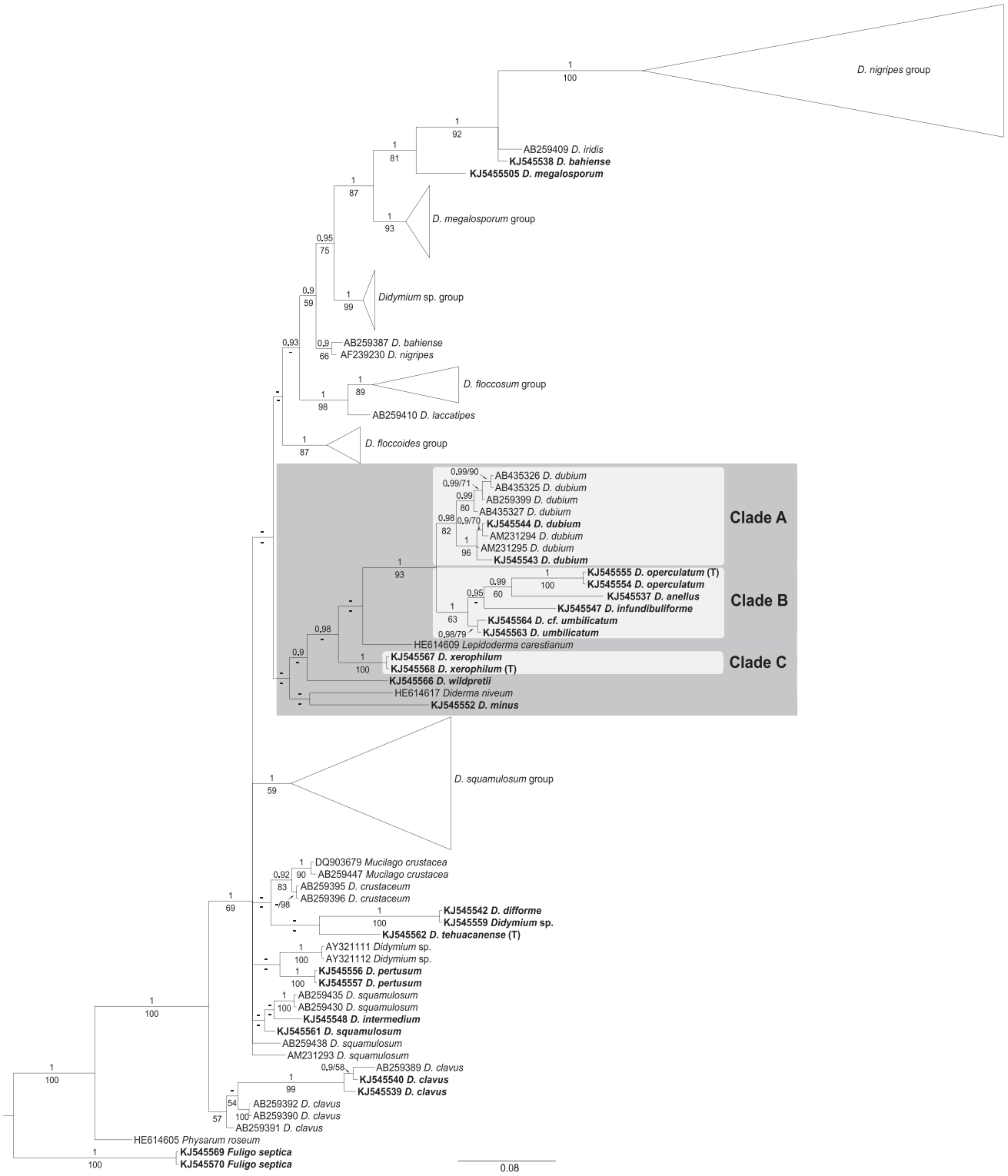
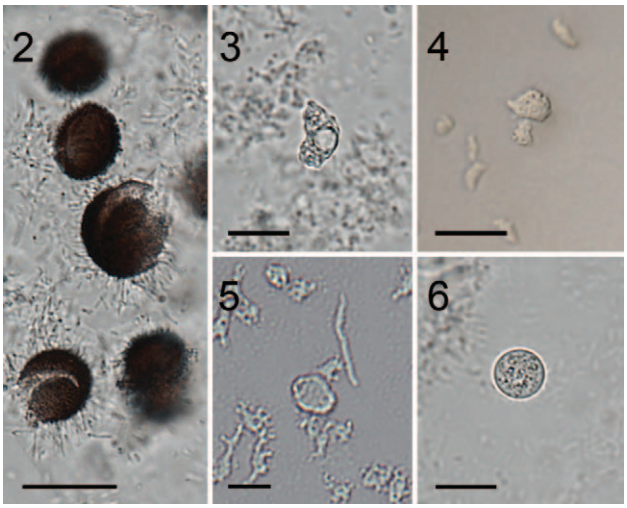


FIG. 1. Nuclear 18S rDNA tree derived from Bayesian inference of 1256 nucleotide positions of 97 sequences, with *Fuligo septica* and *Physarum roseum* as outgroup. The group of interest includes *Didymium xerophilum* sequences and the most closely related taxa and is outlined in dark gray. Arbitrarily named clades (A, B, C) are highlighted in light gray. GenBank accession numbers are followed by species names. Those sequences obtained during this study are in boldface. Bayesian posterior probabilities (PP) and maximum likelihood bootstrap support (BS) values are shown for each node (above and below the lines, respectively). Dashes indicate PP < 0.9 and BS < 50. Scale bar indicates the fraction of substitutions per site.



FIGS. 2–6. Stages in the life cycle (isolate Lado 22243, MA-Fungi 86885). 2. Germination of the spore. 3. Amoeba. 4. Zygote. Scale bar = 15 μm. 5. Early plasmodium on agar. Scale bar = 50 μm. 6. Cyst. Scale bar = 15 μm.

Hypothallus concolorous with the stalk or paler, conspicuous, calcareous, with rhomboid lime crystals like the stalk, sometimes venulose, common to several sporocarps (FIGS. 7, 8). Peridium single, membranous, light yellow (73. p. OY–86. l. Y) by LM, its inner surface smooth by SEM (FIG. 16), covered with abundant to scattered, white, stellate, lime crystals (FIGS. 7–11, 15); dehiscence circumscissile near the apex of the sporotheca, leaving a persistent funnel-shaped invagination that reaches the columella (FIGS. 9–11). Columella from a disk-shaped thickening to dome-shaped, packed with lime crystals, continuous with the stalk (FIGS. 11–12, 15), concolorous with the stalk or slightly darker. Capillitium filiform, netted, threads 0.5–1.5 μm diam, branched, straight, with many cross connections with small expansions, yellow-brown (79. l. gy. y Br–78. d. y Br), colorless at the tip, covered with nodules by LM and SEM (FIGS. 14, 17–18). Spores free, black in mass, dark brown (78. d. y Br–81. d. gy. Y Br) by LM, with a pale area, globose, (10–)11–13(–15) μm diam, densely warted by LM (FIG. 2), with evenly distributed bacula by SEM (FIGS. 19–20). Plasmodium not observed.

Habitat: Leaf litter and twigs of arid plants and blades of grasses.

Distribution: Argentina (Jujuy, Neuquen, Chubut) and Peru (Arequipa, Ayacucho, Moquegua).

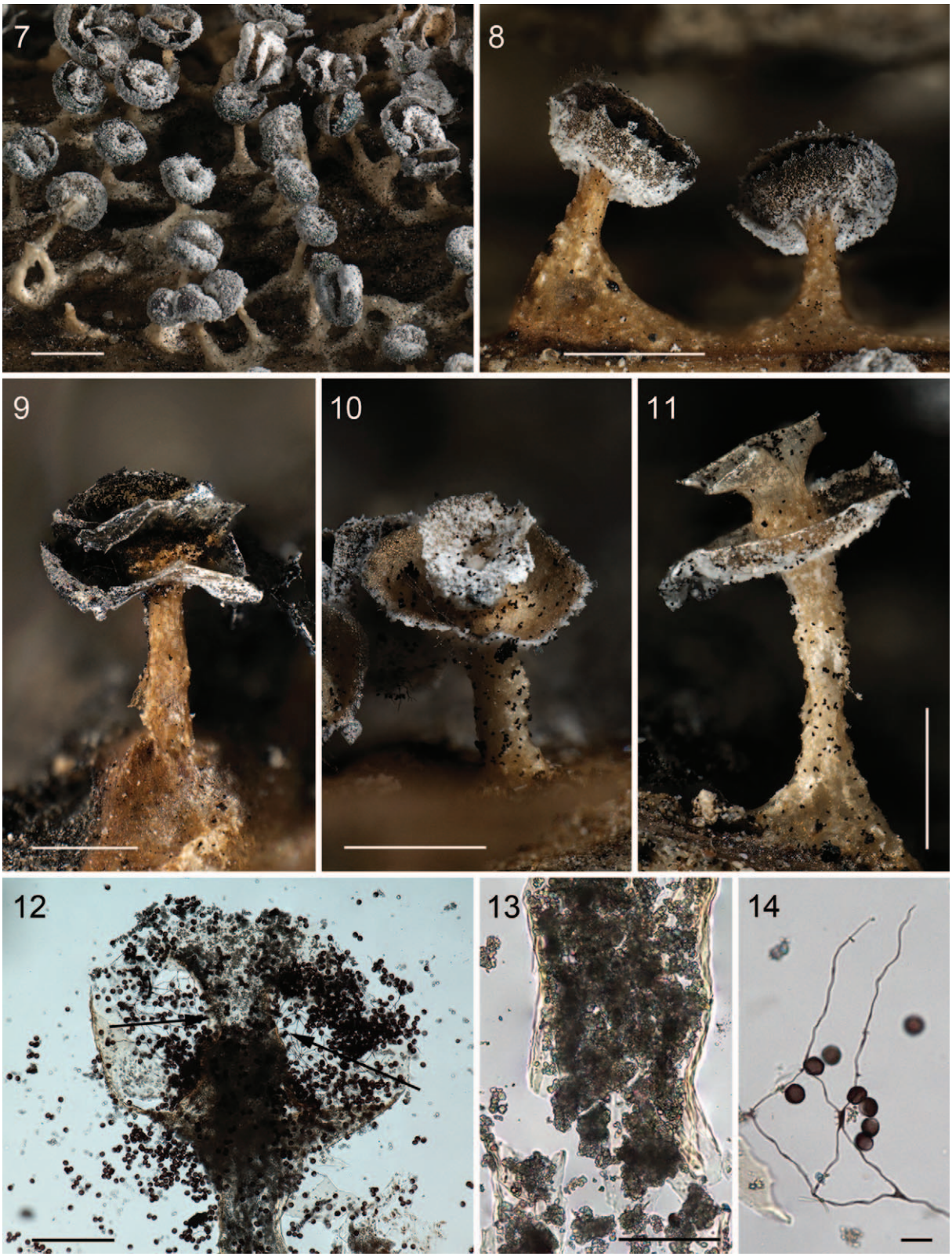
Other specimens examined: ARGENTINA. Jujuy: Tumbaya, Purmamarca, El Quemado, route RN-52, near Abra de Potrerillos, 23°41′39″S, 65°38′58″W, 4149 m, on *Stipa atacamensis*, 20 Nov 2006, *Lado 18138* (MA-Fungi 86878), *Lado 18140* (MA-Fungi 86879). Neuquén: Huiliches, Junín de los Andes, route RN-40, km 2226, southeast of La Rinconada, 40°01′02″S, 70°49′24″W, 668 m, on *Eryngium paniculatum* leaves, 5 Nov 2009, *Lado 20262* (MA-Fungi 86880), *Lado 20274* (MA-Fungi 86877), *Lado 20276* (MA-Fungi 86881). Chubut: Paso de Indios, Los Altares, route RN-25, km 292, 43°50′26″S, 68°11′28″W, 228 m, on *Cortaderia* sp., 16 Nov 2009, *Lado 20410* (MA-Fungi 86882). PERU. Ayacucho: Lucanas, Abra Condorcenca, route PE-30A, km 99, 14°39′39″S, 74°18′43″W, 4130 m, on leaf litter of an Asteraceae, 27 Sep 2012, *Lado 21888* (MA-Fungi 86883). Moquegua: Mariscal Nieto, Torata, route PE-34D, km 76, 16°58′45″S, 70°41′29″W, 4130 m, on leaf litter of *Polylepis besseri*, 7 Oct 2012, *Lado 22188* (MA-Fungi 86896); on *Polylepis besseri* bark, *Lado 22189* (MA-Fungi 86897), *Lado 22192* (MA-Fungi 86898). Chillihua, route PE-34D, km 88, 16°54′55″S, 70°38′52″W, 4580 m, on *Festuca orthophila,* 7 Oct 2012, *Lado 22202* (MA-Fungi 86889). Arequipa: Caylloma, San Antonio de Chuca, route PE-34A, km 157, 18 km west of Imata, 15°52′40″S, 71°08′54″W, 4444 m, on leaf litter and twigs of *Parastrephia phyliciformis*, 8 Oct 2012, *Lado 22241* (MA-Fungi 86884), *Lado 22244* (MA-Fungi 86886), *Lado 22245* (MA-Fungi 86887), *Lado 22247* (MA-Fungi 86888). Arequipa, Yura, Cañahuas, route PE-1SE to Chivay, km 9, 15°59′13″S, 71°23′04″W, 4230 m, on *Parastrephia lepidophylla* twigs, 9 Oct 2012, *Lado 22276* (MA-Fungi 86890), *Lado 22277* (MA-Fungi 86891); on *Stipa ichu, Lado 22283* (MA-Fungi 86892). Arequipa, Chiguata, route PE-34C, km 30, west of Chiguata, 16°23′35″S, 71°19′07″W, 4053 m, on *Polylepis besseri* bark, twigs and leaves, 10 Oct 2012, *Lado 22344* (MA-Fungi 86893), *Lado 22359* (MA-Fungi 86894), *Lado 22360* (MA-Fungi 86895).

Examples of other species examined are provided (TABLE III).

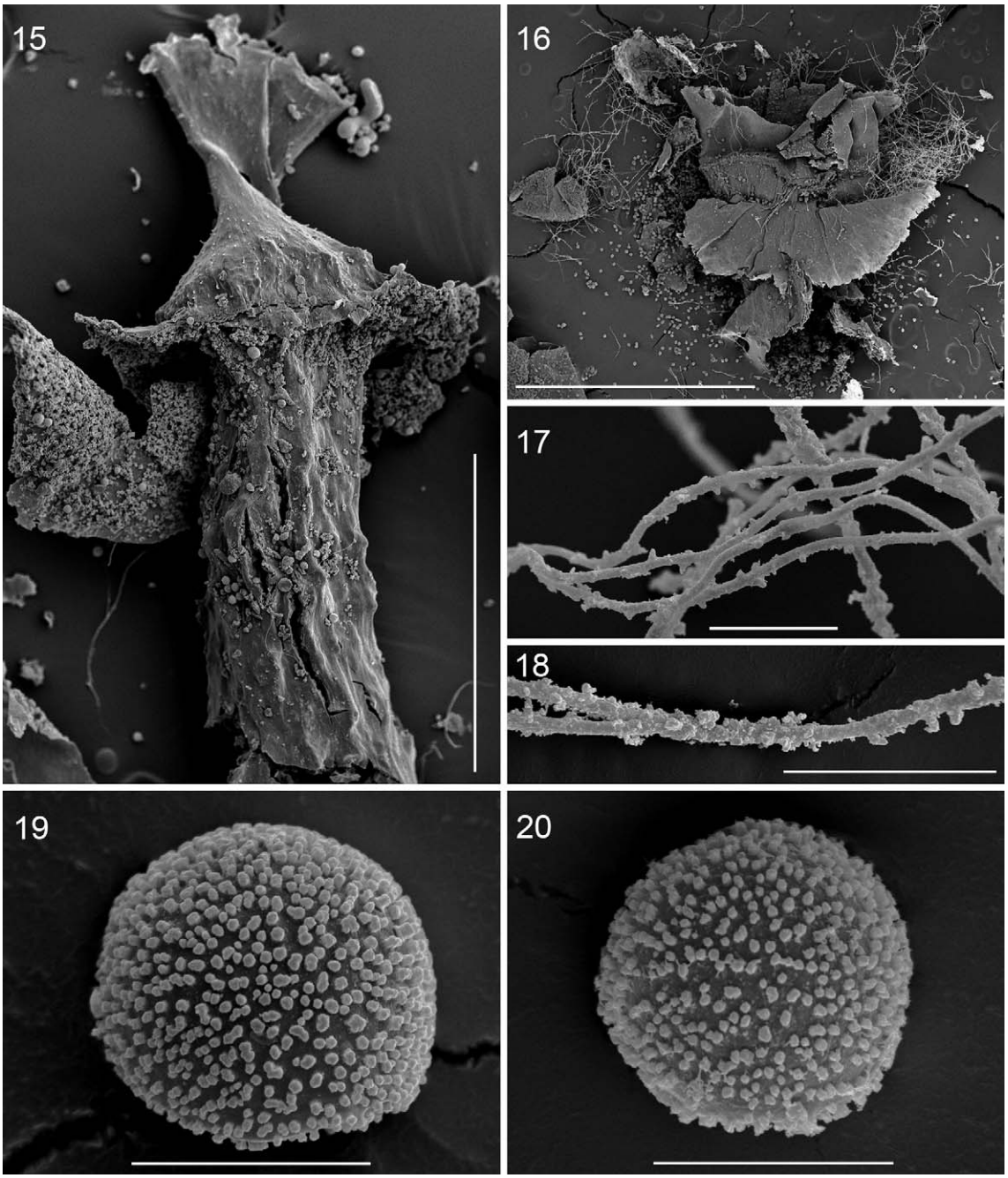
DISCUSSION

Twenty-one collections of this new species were obtained from two countries in three separate years. Some stages of the life cycle of this myxomycete also were observed on agar from the germination of spores produced by natural fruiting in the field. The phylogenetic analysis of nuc 18S rDNA sequences shows the relationship of the new species to others in the genus. In addition, clear morphological differences separate it from currently described species.

The most obvious characters that make this species unique in *Didymium* are the persistent funnel-shaped invagination of the peridium, the top of which appears as a deep umbilicus in closed sporothecae, and the vein-like calcareous hypothallus common to several sporocarps. The combination of these characters with the circumscissile dehiscence and the yellowish white stalk packed with rhombic lime crystals is unknown in any other described species. The only other member of *Didymium* with a funnel-shaped invagination of the peridium is the recently described *Didymium infundibuliforme* D. Wrigley, Lado & Estrada, but the new species differs in several aspects. When compared with the type material of *D.*



FIGS. 7–14. *Didymium xerophilum*. 7–8. (Lado 20274, MA-Fungi 86877). 7. Habit. Scale bar = 1 mm. 8. Sporocarps showing a common calcareous hypothallus. Scale bar = 1 mm. 9. (Holotype, Lado 22243, MA-Fungi 86885). Detail of dehisced sporotheca. Scale bar = 1 mm. 10–11. (Lado 20274, MA-Fungi 86877). Dehisced sporocarps showing the remains of the peridium and the columella. Scale bar = 0.5 mm. 12–14. (Lado 22247, MA-Fungi 86888). 12. Section of mature sporocarp showing details of the sporotheca and the peridium connected to the columella. Scale bar = 200 µm. 13. Detail of the stalk packed inside with rhomboid lime crystals. Scale bar = 100 µm. 14. Capillitial threads. Scale bar = 20 µm.



FIGS. 15–20. *Didymium xerophilum*. 15. (Lado 22247, MA-Fungi 86888). Detail of stalk, peridium and columella by SEM. Scale bar = 500 µm. 16. (Lado 22243, MA-Fungi 86885). Open sporotheca showing the inner surface of the peridium, the capillitium and the spores by SEM. Scale bar = 1 mm. 17. (Lado 22247, MA-Fungi 86888). Capillitial threads by SEM. Scale bar = 50 µm. 18. (Lado 22243, MA-Fungi 86885). Detail of a capillitial thread covered with nodules by SEM. Scale bar = 20 µm. 19. (Lado 22243, MA-Fungi 86885). Spore by SEM. Scale bar = 10 µm. 20. (Lado 22247, MA-Fungi 86888). Spore by SEM. Scale bar = 10 µm.

infundibuliforme, *D. xerophilum* is much larger (0.75–2 mm vs. 0.2–0.6 mm in *D. infundibuliforme*), has a true columella (absent in *D. infundibuliforme*), the funnel is attached to the columella (to the base of the sporotheca in *D. infundibuliforme*), the hypothallus is conspicuous, calcareous and common to several sporocarps (inconspicuous to absent in *D. infundibuliforme*) and the spores are ornamented with evenly distributed bacula by SEM (with verrucae and bacula interconnected at their bases by an irregularly meshed net of bands in *D. infundibuliforme*). In addition, the sporotheca is obconical in *D. infundi-*

TABLE III. Data on examined specimens of other species

Specimen	Collection No.	Locality	Substrate
D. infundibuliforme D. Wrigley, Lado & Estrada	MA-Fungi 78320 Holotype	ARGENTINA. Catamarca: Tinogasta, Costa de Reyes, road RP-3	leaf of *Puya* sp.
D. operculatum D. Wrigley, Lado & Estrada	MA-Fungi 74050 Holotype	CHILE. Region II Antofagasta: Antofagasta, route RN-1, Blanco Encalada	epidermis of dead *Copiapoa* sp.
D. reticulosporum Novozh. & Zemly.	LE 220334 Isotype	RUSSIA. Volgograd province, Otrada wash, Ergeni, the watershed of the Volga River	dead twigs of *Artemisia lerchiana*
D. tehuacanense Estrada, D. Wrigley & Lado	MA-Fungi 73605 Holotype	MEXICO. Puebla: Puebla,Tehuacán-Cuicatlán Biosphere Reserve, San Martín Esperilla	leaf of *Agave* sp.
D. umbilicatum D. Wrigley, Lado & Estrada	MA-Fungi 73566 Holotype	MEXICO. Querétaro, Peñamiller, Plazuela	*Yucca* sp.

buliforme (Wrigley de Basanta et al. 2009) and slightly oblate to subhemispheric in the new species.

Didymium xerophilum can be distinguished easily from other *Didymium* species with calcareous stalks because none have the funnel-shaped invagination of the peridium of the new species. In addition, *Didymium squamulosum* (Alb. & Schwein.) Fr. & Palmquist does not have a stalk packed with rhomboid lime crystals (Martin and Alexopoulos 1969), *D. laccatipes* Matsumoto has strongly warted spores, often with dark clusters of warts (Matsumoto and Deguchi 1999), *D. floccosum* G.W. Martin, K.S. Thind & Rehill has spores with warts arranged in a reticulate pattern (Martin et al. 1959), and *D. intermedium* J. Schröt. has spores with long, dark spines, also in a partial reticulate pattern (Martin and Alexopoulos 1969). *Didymium wildpretii* Mosquera, Estrada, Beltran-Tej., D. Wrigley & Lado, also has smaller spores (7.5–9.5 μm diam vs. 11–13 μm) with warts in a subreticulate pattern and shorter stalks (0.1–0.3 mm vs. 0.25–1.5 mm). *Didymium pertusum* Berk. has a slightly tapering reddish stalk and is umbilicate below, not above. In addition, none except *D. intermedium* has the calcareous, sometimes venulose hypothallus, common to several sporocarps (Fries and Palmquist 1818, Berkeley 1836, Martin et al. 1959, Matsumoto and Deguchi 1999, Lado et al. 2007b).

Didymium operculatum D. Wrigley, Lado & Estrada has a circumscissile dehiscence, but the top of the sporotheca lifts off as a lid. It also has reticulate spores, 10–11 μm diam, with 9–12 meshes across the hemisphere, unwarted spores, with bacula by SEM as in *D. xerophilum*. *Didymium tehuacanense* Estrada, D. Wrigley & Lado can be distinguished from the new species because it is not umbilicate, has irregular dehiscence, leaving a basal disk, and has an inconspicuous membranous orange brown hypothallus. This species also has smaller spores (8–10 μm diam vs. 11–13 μm) (Estrada-Torres et al. 2009). *Didymium umbilicatum* D. Wrigley, Lado & Estrada has an umbilicus above, but the sporocarps are flattened sessile or with very short stalks, the hypothallus is inconspicuous and membranous, and the spores have an irregular sub-reticulum of warts by SEM (Wrigley de Basanta et al. 2008).

A species of *Physarum* that is macroscopically similar to *D. xerophilum* is *P. umbiliciferum* Y. Yamam. & Nann.-Bremek., because it has a deep umbilicus, but the dense net of the physaroid capillitium easily distinguishes it from the new *Didymium*. *Physarum umbiliciferum* also has a pale brown discoid hypothallus and smaller spores (8–10 μm diam vs. 11–13 μm), and the umbilicus does not reach the base of the sporotheca or columella, which is absent (Nannenga-Bremekamp and Yamamoto 1990).

As illustrated (FIG. 1) both nuc 18S rDNA sequences of *Didymium xerophilum*, newly generated in this study, constituted a well supported monophyletic clade (clade C; PP = 1, BS = 100%). These two sequences were identical, despite the origin of the specimens from geographically distant areas (TABLE I), suggesting low intraspecific genetic variation. This fact, together with the unique morphology of *D. xerophilum*, supports the distinct identity of this new species. This analysis also shows that different members of the genus *Didymium*, which share some morphological traits with *D. xerophilum*, are phylogenetically separated. For instance, *D. infundibuliforme*, which also has a funnel-shaped invagination in the peridium, forms clade B with *D. operculatum*, *D. anellus* and *D. umbilicatum* (PP = 1, BS = 63%). Similarly *D. operculatum* has a calcareous stalk like the new species but is placed closer to *D. anellus*, which lacks this character. Other taxa, such as *D. floccosum, D. laccatipes* and *D. squamulosum*, which could be thought to be allied to *D. xerophilum*

because they have some morphological similarities, stand apart in clearly separate clades with either moderate or strong support.

Ecology.—Many species of *Didymium* appear to be particularly xerotolerant, and members of the genus that are commonly found in or associated with arid conditions now number more than 25 (Wrigley de Basanta et al. 2011). *Didymium xerophilum* appears to be a generalist because it was found on several substrate plants from the genera *Cortaderia, Eryngium, Festuca, Parastrephia, Polylepis* and *Stipa*. These are characteristic plants in cold, arid areas of either puna or steppe vegetation of South America. The new species was collected at markedly different elevations, from 228 m to almost 4600 m, one of the highest reported for a myxomycete, but always from dryland ecosystems. The presence of a previously unknown species of microorganisms in such vulnerable areas of the tropical Andes suggests a further pressing reason for the careful conservation and protection of these environments.

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LITERATURE CITED

Anonymous. 1976, ISCC-NBS color-name charts illustrated with centroid colors. Washington: Inter-Society Color Council, National Bureau of Standards.

Berkeley MJ. 1836. Fungi. In: Smith JE, ed. The English flora. London: Longman, Hurst, Rees, Orme, Brown and Green Vol. 5. p 1–386, doi:10.5962/bhl.title.6340

Brown JM, Hedtke SM, Lemmon AR, Lemmon EM. 2010. When trees grow too long: investigating the causes of highly inaccurate Bayesian branch-length estimates. Syst Biol 59:145–161, doi:10.1093/sysbio/syp081

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772, doi:10.1038/nmeth.2109

Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A. 2010. Geneious 5.0, available from http://www.geneious.com

Estrada-Torres A, Wrigley de Basanta D, Conde E, Lado C. 2009. Myxomycetes associated with dryland ecosystems of the Tehuacán-Cuicatlán Valley Biosphere Reserve, Mexico. Fungal Divers 36:17–56.

Fiore-Donno AM, Meyer M, Baldauf SL, Pawlowski J. 2008. Evolution of dark-spored myxomycetes (slime molds): molecules versus morphology. Mol Phylogenet Evol 46: 878–889, doi:10.1016/j.ympev.2007.12.011

Fries EM, Palmquist LG. 1818. Symbolae gasteromycorum as illustrandam floram suecicam. Acad Carol Fasc 3:17–25.

Haskins E, Wrigley de Basanta D. 2008. Methods of agar culture of myxomycetes—an overview. Rev Mex Micol 27:1–7.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755, doi:10.1093/bioinformatics/17.8.754

Kamono A, Fukui M. 2006. Rapid PCR-based method for detection and differentiation of Didymiaceae and Physaraceae (myxomycetes) in environmental samples. J Microbiol Methods 67:496–596, doi:10.1016/j.mimet.2006.05.003

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30: 3059–3066, doi:10.1093/nar/gkf436

Lado C. 2005–2014. An online nomenclatural information system of Eumycetozoa. http://www.nomen.eumycetozoa.com (28 Jul 2014).

———, Estrada-Torres A, Stephenson SL. 2007a. Myxomycetes collected in the first phase of a north-south transect of Chile. Fungal Divers 25:81–101.

———, Mosquera J, Estrada-Torres A, Beltran-Tejera E, Wrigley de Basanta D. 2007b. Description and culture of a new succulenticolous Didymium (Myxomycetes). Mycologia 99:602–611, doi:10.3852/mycologia.99.4.602

———, Wrigley de Basanta D, Estrada-Torres A. 2011. Biodiversity of Myxomycetes from the Monte Desert of Argentina. An Jard Bot Madr 68:61–95, doi:10.3989/ajbm.2266

———, ———, ———, Stephenson SL. 2013. The biodiversity of myxomycetes in central Chile. Fungal Divers 59:3–32, doi:10.1007/s13225-012-0159-8

Larget B, Simon D. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol Biol Evol 16:750–759, doi:10.1093/oxfordjournals.molbev.a026160

Lutzoni F, Kauff F, Cox C, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James T, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung GH, Lücking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hansen K, Harris RC, Hosaka K, Lim YW, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R. 2004. Assembling the fungal tree of life: progress, classification and evolution of subcellular traits. Am J Bot 91:1446–1480, doi:10.3732/ajb.91.10.1446

Martin GW, Alexopoulos CJ. 1969. The Myxomycetes. Univ Iowa Press. 561 p, doi:10.1126/science.169.3949.966

———, Thind KS, Rehill PS. 1959. The myxomycetes of the Mussoorie Hills (India) X. Mycologia 51:159–162, doi:10.2307/3756209

Matsumoto J, Deguchi H. 1999. Two new species of *Didymium* (Physarales, Myxomycetes) from Japan. Mycotaxon. 70:153–161.

Nandipati SCR, Haugli K, Coucheron DH, Haskins EF, Johansen SD. 2012. Polyphyletic origin of the genus *Physarum* (Physarales, Myxomycetes) revealed by nuclear rDNA mini-chromosome analysis and group I intron synapomorphy. BMC Evol Biol 12:166, doi:10.1186/1471-2148-12-166

Nannenga-Bremekamp NE, Yamamoto Y. 1990. Additions to the Myxomycetes of Japan IV. Proc K Ned Akad Wetensch, Ser C 93:265–280.

Rambaut A. 2013. FigTree 1.4., available at http://tree.bio.ed.ac.uk/software/figtree

———, Suchard MA, Xie D, Drummond AJ. 2013. Tracer 1.5., available from http://beast.bio.ed.ac.uk/Tracer

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542, doi:10.1093/sysbio/sys029

Schrader HA. 1797. Nova genera plantarum. Pars prima. Lipsiae, doi:10.5962/bhl.title.12910

Silvestro D, Michalak I. 2012. RAxMLGUI: a graphical front-end form RAxML. Org Divers Evol 12:335–337, doi:10.1007/s13127-011-0056-0

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690, doi:10.1093/bioinformatics/btl446

Wrigley de Basanta D, Lado C, Estrada-Torres A. 2008. Morphology and life cycle of a new species of *Didymium* (Myxomycetes) from arid areas of Mexico. Mycologia 100:921–929, doi:10.3852/07-168

———, ———, ———. 2010. *Licea eremophila* a new Myxomycete from arid areas of South America. Mycologia 102:1185–1192, doi:10.3852/09-309

———, ———, ———. 2011. Spore to spore culture of *Didymium operculatum,* a new Myxomycete from the Atacama Desert of Chile. Mycologia 103:895–903, doi:10.3852/10-363

———, ———, ———. 2012. Description and life cycle of a new *Physarum* (Myxomycetes) from the Atacama Desert in Chile. Mycologia 104:1206–1212, doi:10.3852/11-427

———, ———, ———, Stephenson SL. 2009. Description and life cycle of a new *Didymium* (Myxomycetes) from arid areas of Argentina and Chile. Mycologia 101:707–716, doi:10.3852/08-227

Zamora JC, Calonge FD, Hosaka K, Martín MP. 2014. Systematics of the genus *Geastrum* (Basidiomycota, Fungi) revisited. Taxon 63:477–497, doi:10.12705/633.36