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Description and life cycle of a new *Physarum* (Myxomycetes) from the Atacama Desert in Chile

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Abstract: A new species of Physarum (Myxomycetes), *Physarum atacamense* is described in this paper, and details are provided on its life cycle as observed in spore-to-spore culture in agar. The new species was collected 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 combination of characters that make this species unique in the genus are its large fusiform nodes of the capillitium, its long, bicolored stalk and the very dark brown and densely warted angular spores. The morphology of specimens of this myxomycete was examined with scanning electron microscopy and light microscopy, and micrographs of relevant details and life cycle stages are included in this paper. The importance of resistant stages in the life cycle of this myxomycete is stressed, and the close association of this myxomycete with its plant substrates is discussed.

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

Introduction

During a biodiversity survey of northern Chile in 2008, as part of a recent research effort to study the myxomycetes of arid areas of the Neotropics (Lado et al. 2007, 2009, 2011; Estrada-Torres et al. 2009; Wrigley de Basanta et al. 2010, 2011), several collections of a myxomycete of the genus *Physarum* were made in the Atacama Desert. Specimens of this taxon were collected in the field, isolated from moist chamber cultures and cultured from spore-to-spore on agar. Because they do not correspond to any described species and constant morphological characters have been maintained in laboratory culture it is proposed here as a new species. The genus *Physarum* is the largest and one of the oldest genera of Myxomycetes, described by Persoon (1794) based on *Physarum aureum* Pers., a synomym of the well known *P. viride* (Bull.) Pers. The genus now includes approximately 140 species (Lado 2005–2011).

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Materials and methods

The southern limit of the Atacama Desert, 24–26°S and 70°W, was the area pertinent to this paper. It is discussed in Lado et al. (2012). The vegetation of the study area was predominantly xerophilous scrubland with species of the globose cacti of the endemic genus *Copiapoa* and the bromeliad *Puya* 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, then dried. Material for preparation of moist chamber cultures also was air-dried in situ and transported to the laboratory in sealed paper bags. All localities were geo-referenced with a GPS monitor (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 3059 and dwb 3470 from moist chamber culture (mc). Spores were sown on 0.75% water agar (WA) at pH 7.0. The sporocarps were crushed and spores released over the agar in each of four quadrants of sterile 9 cm plastic Petri dishes. Slide cultures for microphotography of germination and myxamoebae were made according to the technique described by Spiegel et al. (2005). A sterile nutrient solution was made from 25 g substrate (*Copiapoa* remains) in 1 L distilled water to be added to germination and early growth plates and germination slides. Agar cultures were kept in an incubator at 23 C, with an approximate 12 h light-dark regime. Further details of the media used and techniques followed can be found in Haskins and Wrigley de Basanta (2008).

Specimens are deposited in herbaria MA-Fungi (sub Lado), TLXM (sub aet) and private collection of the first author (dwb). Microscope measurements and observations were made with material mounted directly in Hoyer's medium. A microscope with differential interference contrast (DIC) was used to obtain descriptive data and light micrographs. Measurements were made of 10 spores from each of the collections. The critical-point drying technique was used for scanning electron microscope (SEM) preparations, and the SEM analyses and photomi-

crographs of specimens were done with a Hitachi S-3000N microscope at 10–15 kV, at the scanning electron microscopy service of the Royal Botanic Gardens of Madrid. Color notations in parentheses are from the ISCC-NBS Color name charts illustrated with centroid colors (Kelly and Judd 1972). Dates are given when the field collections and substrate material were obtained (first date) and when samples were obtained from moist chamber cultures (second date).

RESULTS

TAXONOMY

Physarum atacamense D. Wrigley, Lado et Estrada, sp. nov. FIGS. 1–24

MycoBank: MB 564038

Sporophores sporocarpic, erect, scattered to grouped. Sporocarps stipitate, 0.3–1.1 mm high. Sporotheca subhemisphaerical to subglobose, or reniform to multilobate due to fusion of the sporocarps, 0.2–0.8(–1.2) × 0.1–0.4 mm, (FIGS. 1, 2), whitish (263. White - 264. l. Gray). Hypothallus membranous, circular under individual sporocarps, occasionally several fused, dark brown (78. d. y Br) with refuse material. Stalk erect, yellow (86. l. Y – 89. p. Y), usually dark yellowish brown (78. d. Y - 88. d- Y – 94. l. Ol Br) at the base in reflected light, by transmitted light yellowish brown (72. d. OY – 74. s. y Br) at the base, not calcareous, cylindrical, ribbed (FIG. 5), 0.15–0.8 mm high, 0.05–0.1 (–0.2) mm wide, sometimes attenuating to the apex. Peridium thin single, membranous, usually heavily spotted with lime, colorless by LM; dehiscence irregular. Columella absent. Capillitium composed of white elongated nodes, 20–30 μm long, joined into a network of fine limeless threads (FIGS. 4, 5, 7), visible in dehisced sporocarps, still attached to the peridium. Spores black in mass, dark brown to blackish (61. gy. Br – 62. d. Gy. Br – 65. br Black) by LM, subglobose, somewhat angular, (11–)12–15(–16) μm diam, evenly and densely warted by LM (FIG. 3), with dense baculate ornamentation by SEM (FIGS. 6, 8). Plasmodium phaneroplasmodium, translucent becoming white (FIGS. 20–24).

Holotype. CHILE. Antofagasta Region II, Antofagasta, route RN-1, Caleta Colorada, 49 km north of Paposo, 24°41′53″S 70°33′43″W, 36 ± 7 m, xerophyllous scrubland with *Copiapoa*,sp., *Puya* sp., *Euphorbia* sp. and *Eulychnia* sp., 18-II-2008, on *Puya* sp. leaf bases obtained in moist chamber culture, 21-V-2008, pH 6.7 *D. Wrigley de Basanta*, *dwb 3059* (MA-Fungi 81399).

Other specimens examined. CHILE. Antofagasta Region II, Antofagasta, Caleta Colorada, 49 km north of Paposo, 24°41′53″S 70°33′43″W, 36 ± 7 m, xerophyllous scrubland with *Copiapoa* spp., *Puya* sp. *Euphorbia* sp. and *Eulychnia* sp., 18-II-2008, on decayed remains of *Copiapoa* sp., MA-Fungi 88261 (*Lado 19085*), MA-Fungi 88262 (*Lado 19086*), MA-Fungi 88263 (*Lado 19087*), MA-Fungi 88264 (*Lado 19088*), MA-Fungi 88265 (*Lado 19089*); on dead leaf bases of *Puya* sp. in mc, 19-V-2008, *dwb 3050*, pH 6.8; 25-V-2008, *dwb 3085*, pH 6.3; on *Copiapoa* sp. epidermis in mc, 12-X-2008, *dwb 3106*, pH 6.9; *dwb 3128*, pH 7.3; *dwb 3130*, pH 7.4. Route RN-1, Blanco Encalada, 24°25′57″S 70°31′59″W, 137 ± 7 m, xerophyllous scrubland with *Copiapoa* sp., 18-II-2008, on *Copiapoa* sp. epidermis in mc, 28-II-2009, *dwb 3160*, pH 7.1. Route RN-1, from Taltal to Paposo, km 41, 25°16′08″S 70°26′16″W, 45 ± 9 m, xerophyllous scrubland with *Copiapoa* sp., 19-II-2008, on decayed remains of *Copiapoa* sp., MA-Fungi 88266 (*Lado 19090*), MA-Fungi 88267 (*Lado 19093*), MA-Fungi 88268 (*Lado 19094*), MA-Fungi 88269 (*Lado 19095*). Route RN-1, from Paposo to Caleta Rincón, 24°57′44″S 70°28′35″W, 36 ± 7 m, xerophyllous scrubland, with *Copiapoa* sp., *Eulychnia* sp. and *Euphorbia* sp. 19-II-2008, on decayed remains of *Copiapoa cinerascens* in mc, 26-X-2010, pH 8.7, *aet-12145*; 30-X-2010, pH 8.6, *aet-12150*. Atacama Region III, Chañaral, route to Pan de Azúcar NP, km 12, 26°17′48″S 70°37′53″W, 20 ± 6 m, xerophyllous scrubland with *Copiapoa* sp., 16-II-2008, on decayed remains of *Copiapoa cinerascens* in mc, 19-V-2011, pH 8.2, *dwb 3467*; 28-V-2011, pH 8.4, *dwb 3468*; 14-V-2011, pH 7.9, *dwb 3469*; 2-X-2011, pH 8.3, *dwb 3470*.

On agar culture of spores from collection *dwb 3059* (holotype) isolates *dwb 1125*, *1126*, *1128*.

Physarum nigripodum Nann.-Bremek. & Y. Yamam., *NENB14806* (BR-MYCO 67669-60),(isotype). On the bark of *Ginkgo biloba*. Higashi-hachimangu. Aki shi, Kochi Pref., 24-VI-1985.

Etymology. The epithet *atacamense* refers to the geographic area where the species was found.

Habitat. On the dead remains of the cactus *Copiapoa* spp., and leaf bases of *Puya* sp.

Distribution. Known only from the Atacama Desert, northern Chile (Antofagasta and Atacama regions).

DISCUSSION

Taxonomy.—Twenty-five collections have been made of this new myxomycete. Of these nine were natural fruitings in the field and 13 were obtained from moist chamber cultures. An additional three collections resulted from agar cultures. The myxomycete has appeared either on species of the endemic cactus genus *Copiapoa* or on the remains of the bromeliad *Puya*, from five localities in two regions of Chile. The life cycle has been observed from spore-to-spore on artificial media, and the characters have remained constant in all of these collections. The major distinguishing features of the species are the erect, stipitate sporocarps with a non-calcareous fibrous stalk, usually dark yellowish brown at the base and paler at the top, the large, white, elongated lime nodes of the capillitium with a network of limeless

FIGS. 1–9. 1, 3, 5–7. *Physarum atacamense* (Holotype dwb 3059, MA-Fungi 81399). 1. Habit. Bar = 1 mm. 2, 4, 8. *Physarum atacamense* (dwb 3106). 2. Sporocarp showing capillitium and bicolored stalk. Bar = 1 mm. 3. Spores by light microscope. Bar = 10 µm. 4. Capillitium by SEM. Bar = 20 µm. 5. Whole dehisced sporocarp by SEM. Bar = 200 µm. 6. Spore by SEM showing angular shape and dense ornamentation. Bar = 10 µm. 7. Capillitium and spore by SEM. Bar = 20 µm. 8. Spores by SEM showing baculate ornamentation. Bar = 10 µm. 9. *Physarum nigripodum* NENB 14806 (isotype) spore by SEM. Bar = 10 µm.

narrow threads between them, and the dark brown, angular, densely warted spores.

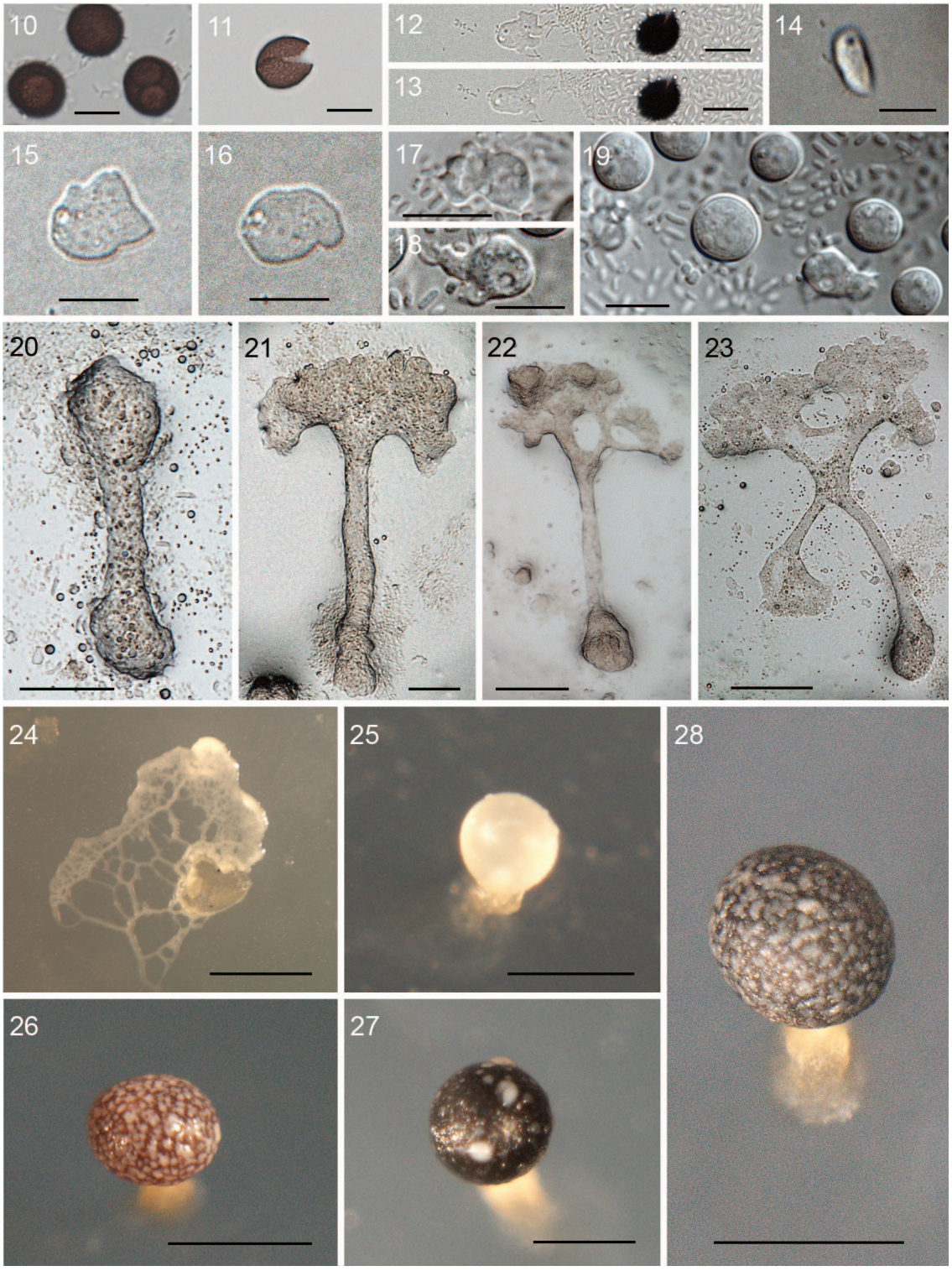
The closest described species to *Physarum atacamense* is *Physarum nigripodum* Nann.-Brem. & Y. Yamam., but this species has a stalk that is calcareous above and black and opaque below (Nannenga-Bremekamp and Yamamoto 1987). We examined the type specimen, and it has a thick opaque cylindrical stalk and a double peridium. It also can be distinguished from *P. atacamense* by the small, rounded lime nodes and spores that are not angular, are paler and have a less dense pilate ornamentation by SEM (FIG. 9). Another species with a bicolored stalk is *P. caesiellum* Chao H. Chung & Tzean, but it has a saucer-like base to the sporotheca, and is gray to light green with pale yellowish brown spores, 9.1–10.4 μm diam, that are minutely warted (Chen and Li 1998). *Physarum alvoradianum* Gottsb., has a black and lime-encrusted stipe, with spores 9.2–10.4(–11.5) μm diam, but the stipe is rugose, the spores have groups of warts and the sporocarps have a discoid pseudocolumella (Gottsberger 1968, Lado et al. 2003). *Physarum album* (Bull.) Chevall. differs from the new species because it has sporocarps that are usually pendulous and a delicate capillitium radiating from the base, not a network of limeless narrow threads joining large white elongated lime nodes, and smaller, paler spores (Poulain et al. 2011).

The new species can be distinguished from some other stalked white species that have been described recently because they all have spores of less than 11 μm diam not 12–15 μm such as *Physarum atacamense*. These are *P. confusum* Shuang L. Chen & Yu Li, *P. florigerum* (Meyl.) Y. Yamam., *P. myricanum* Y. Yamam., *P. taiwanianum* Chao H. Chung & C.H. Liu and *P. scoticum* Ing. (Chen and Li 2000; Yamamoto 1994, 2000; Chung and Liu 1996; Ing 1982). The last species also differs in its calcareous stalk.

Physarum spectabile Nann.-Bremek., Lado & G. Moreno and *Badhamia melanospora* Speg. are species with angular spores and appear on succulent substrates. The first can be distinguished easily from *P. atacamense* because it usually is sessile, but even the stipitate sporocarps (Nannenga-Bremekamp et al. 1984, Lado et al. 2007, Estrada-Torres et al. 2009) have a short white or yellowish stalk, arising as a prolongation of the hypothallus, not a well defined erect and ribbed stalk as in the new species, and the spores have pale, narrow bands, absent in *P. atacamense*. *Badhamia melanospora* has a completely different badhamioid capillitium with calcareous tubes in a network, not limeless tubes connecting large lime nodes, and the stalk, when present, is membranous, of uniform color and extending from a hypothallus common to several sporocarps (Castillo et al. 1996, Moreno and Oltra 2010). Taxonomically there are some overlaps between some species of these genera, as discussed by Martin and Alexopoulos (1969), but this new species is a clear example of the description of the genus *Physarum* on account of its type of capillitium (FIGS. 4, 5, 7).

Culture and life cycle.—The new species took 10–23(–34) d to produce mature sporocarps in moist chamber cultures at room temperature (approx. 22 C), although, as mentioned for other myxomycetes in moist chamber culture (Wrigley de Basanta et al. 2010, 2011), it is not known whether the sporocarps came from spores on the substrate, microcysts or small undetected sclerotia, and so the full life cycle might have been longer. The pH of the moist chamber cultures of *Copiapoa* spp. tissue or *Puya* spp. at 24 h was pH 6.3–8.7, mean 7.04. The higher pH was from the cultures with mainly internal tissue of the cactus.

Spores germinated by a V-shape split in the spore wall (FIGS. 11–13) in all four quadrants of germination plates less than 48 h after being placed on 0.75% water agar (WA). In slide cultures the addition of a sterile nutrient medium made from *Copiapoa cinerascens* reduced germination to 21 h (30 h control from same sporocarp with sterile water), and bacteria and yeast, isolated with the spores, grew in large numbers in the nutrient-enriched culture. Many of the spores contained two protoplasts (FIG. 10). The spores produced myxamoebae about 10 μm long (FIGS. 15–19) and many flagellate swarm cells 10–12 μm long (FIG. 14). Small agar blocks, containing myxamoebae and some spores and spore cases, were transferred from the germination plates to fresh plates with weak malt-yeast agar (wMY) or 1.5% WA, with the bacterial flora isolated incidentally with the spores (FIGS. 17–19). The amoebal population on 1.5% WA grew much more slowly than those on wMY. The population of amoebae in the wMY plates grew even more rapidly when drops of the nutrient medium were added, and so did the food organisms. Small, early plasmodia first appeared 7 d after germination. They exhibited slow intermittent cyclosis and ingested myxomycete microcysts and amoebae that can be observed in food vacuoles inside the plasmodia (FIGS. 20, 21) and were seen circulating inside the vacuoles as digestion occurred. Extra food in the form of finely ground oat flour was added to the cultures when plasmodia formed. Larger phaneroplasmodia formed (FIGS. 22, 23) with normal reversible cytoplasmic streaming and turned a milky white (FIG. 24). The first sporocarps formed 18 d after germination in two of the cultures started on wMY



FIGS. 10–28. *Physarum atacamense*. Stages in the life cycle. 10–13. Slide culture (dwb 3470). Bar = 10 µm. 10. Spores before germination showing two protoplasts. 11. Germination by a V-shaped slit in spore. 12, 13. Flow of protoplasm through opening in the spore. 14–19. Unicellular stages (dwb 3059). 14. Flagellated swarm cell. Bar = 20 µm. 15–18. Amoebae showing contractile vacuoles and pseudopodia. Bar = 10 µm. 19. Microcysts with amoeba and bacterial food organisms. Bar = 10 µm. 20. Early plasmodium on agar (isolate dwb 1125). Bar = 100 µm. 21. Plasmodium with early fan formation. Bar = 100 µm. 22–23. Older phaneroplasmodia on agar (isolate dwb 1125). Bar = 200 µm. 24. Phaneroplasmodium with network of fine trailing veins (isolate dwb 1128). Bar = 1 mm. 25–28. Sporocarp morphogenesis. 25. Early sporocarp forming on agar (isolate dwb

(isolates dwb 1125 and dwb 1126) and 36 d in isolate dwb 1128 started on 1.5% WA. They formed as heaps of white plasmodium that elongated perpendicular to the substratum (FIG. 25), most of the protoplasm concentrating at the tip and forming the sporocarp, a subhypothallic development (Ross 1973). The color of the sporocarp darkened from cream to brown (FIG. 26) in a few hours and finally black as the spores developed (FIGS. 27, 28). Lime deposits were seen on the surface of the peridium (FIGS. 26–28) as the sporophores matured and dried. The agar plates were dried slowly with the lid on until the sporocarps were mature.

Ecology.—It has become evident during agar culture studies (Wrigley de Basanta et al. 2008, 2009, 2010, 2011) that myxomycetes from arid areas have evolved a life cycle with a greater dependence on resistant stages. Microcysts (FIG. 19) appear to be widely and frequently used, and in culture amoebae converted to microcysts readily and repeatedly when any unfavorable environmental condition arose. In nature, it is possible that these microcysts are made many times during the unicellular part of the life cycle. Excystment occurs simultaneously in culture, on addition or removal of the limiting factor, usually food or water or accumulation of waste in the closed system conditions of a Petri dish, controlled by unsealing the dish or making subcultures. By forming microcysts in a similar way in their natural environment of rapidly changing conditions such as drying, the myxomonad population could avoid part of the typical r-strategy of a boom/bust population growth curve by halting population growth, even at the peak of the exponential phase, and continuing from the same place when all the microcysts excyst together. This could give them a stepladder-shaped population curve, with the log phase alternating between population increase and periods of stasis repeated many times. Then if it is a viable population, it could pass to the next stage of plasmodial production more rapidly, without starting again from close to zero population. Much more experimental work is necessary to determine whether this is so, and whether it is the case in nature too, but it seems it would be a favorable development for these arid area myxomycetes.

Physarum atacamense is another myxomycete that seems to be associated with a certain plant group. It may be present on other similar substrates, but for example the bromeliad *Puya* species have produced more than 25 species in 130 collections of myxomycetes from moist chamber culture and 155 field collections by the authors, and only three of these collections were of *Physarum atacamense*. Similarly from more than 250 moist chamber cultures of different cacti that have produced 37 species to date, only 10 of these *Copiapoa* spp. cultures have produced the new species. The affinity for cacti of the endemic genus *Copiapoa* as a substrate also has been observed in *Didymium operculatum* (Wrigley de Basanta et al. 2011), and *Licea eremophila* (Wrigley de Basanta et al. 2010) was produced specifically on *Puya* spp. in culture but not from many cultures of a similar bromeliad *Hechtia* spp. from Mexico, a genus with similar morphology but different phylogeny. It appears to be beneficial in culture to introduce a special nutrient from the substrate plant at some point, either to promote germination or to increase the growth of food organisms isolated with the spores. The benefit of some abiotic element(s) from the substrate plant in the form of the nutrient medium also points to a particularly close association between the myxomycete and its substrate plant.

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1128). Bar = 200 μm. 26. Older sporocarp maturing on agar (isolate dwb 1126). Bar = 500 μm. 27. Spores darken in maturing sporocarp (isolate dwb 1128). Bar = 200 μm. 28. Sporocarp drying showing darkened spore mass and lime on the peridium. Bar = 500 μm.

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