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DESCRIPTION AND CULTURE OF A NEW MYXOMYCETE, *LICEA SUCCULENTICOLA*

by

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Resumen

MOSQUERA, J., C. LADO, A. ESTRADA-TORRES, E. BELTRÁN TEJERA & D. WRIGLEY DE BASANTA (2003). Descripción y cultivo de un nuevo mixomicete, *Licea succulenticola*. *Anales Jard. Bot. Madrid* 60(1): 3-10 (en inglés).

Se describe una nueva especie de Myxomycetes, perteneciente al género *Licea*, que se desarrolla en plantas suculentas. Los materiales proceden de España (Islas Canarias), México y Estados Unidos. Se parece a *L. biforis* pero difiere en sus pequeños esporóforos y en sus esporas mayores, amarillo-naranjas, con una gruesa pared que gradualmente hacia un lado se adelgaza y se vuelve más pálida. La estabilidad de estos caracteres ha quedado demostrada por el cultivo de espores a espores en laboratorio.

Palabras clave: Islas Canarias, *Licea*, ciclo vital, ecología, México, España, Myxomycetes succulentícolas, taxonomía, Estados Unidos.

Abstract

MOSQUERA, J., C. LADO, A. ESTRADA-TORRES, E. BELTRÁN TEJERA & D. WRIGLEY DE BASANTA (2003). Description and culture of a new myxomycete, *Licea succulenticola*. *Anales Jard. Bot. Madrid* 60(1): 3-10.

A new succulenticolous myxomycete of the genus *Licea* is described based on material from Spain (the Canary Islands), Mexico and the USA. It is very similar to *L. biforis* but differs in its smaller sporophores, and its bigger orange-yellow spores. The spores have a thick wall that is thinner and lighter towards one side. These differentiating characters were stable in spore to spore culture on agar.

Key words: Canary Islands, *Licea*, life cycle, ecology, Mexico, Spain, succulenticolous myxomycetes, taxonomy, USA.

INTRODUCTION

Licea (Liceales, Myxomycetes) is a genus described in 1797 by Schrader in *Nova Genera Plantarum*, and currently includes more than 65 species (LADO, 2001) from all around the world. More than 80 % of the recognized species have been described in the last three decades, after KELLER & BROOKS (1977) made popular the use of the moist chamber technique to study corticolous species. The minute size of the sporophores (about 0.01-0.5 mm) and the fact that they predominate mainly on the bark of living trees meant that they were easily overlooked. Because of the number of species described recently, and the simplicity of the sporophores, the systematics of the genus has become very controversial, and the limits of the genus with others of different orders, like *Perichaena* (Trichiales), are not clear (GILBERT, 1994).

For more than four years, we have been studying the succulenticolous myxomycetes associated with decaying plant debris in semi-arid lands and deserts in the Canary Islands and Mexico (LADO & *al.*, 1999; MOSQUERA & *al.*, 1999, 2000a, 2000b). During these surveys we have abundantly collected a *Licea* that was initially identified as *L. biforis* but that presents some differences, mainly in the spores. When we compared our material with the type of *L. biforis*, we confirmed the existence of consistent differences. We have also been able to complete its life cycle from spore to spore in the laboratory, verifying the genetic stability of those distinguishing features. Thus we describe it here as a new species.

MATERIALS AND METHODS

This study is partially based on material collected directly in the field on different decaying succulent plants (*Aeonium*, *Agave* and *Opuntia*) from Spain (the Canary Islands), and from Mexico; other samples were obtained in moist chamber cultures of different succulent substrata (*Agave*, *Austrocylindropuntia*, *Euphorbia*, *Opuntia*, *Myrtillocactus*, *Nolina*, *Stenocereus* and *Yucca*), and others from spore to spore cultures on agar.

Moist chamber cultures, were prepared using plastic Petri dishes (90 × 15 mm) fitted with one filter paper disc and little pieces of the substrata. Enough sterile distilled water was added to moisten the contents thoroughly, and the surplus water was removed after one day. Agar cultures were prepared with 2 % oatmeal agar in sterile plastic Petri dishes (90 × 15 mm). Two sporocarps were crushed, and the spores spread on the agar surface and inoculated with a thin layer of distilled water. Plates were incubated in the dark at room temperature and checked daily.

The material studied has been deposited in the herbaria TFC Mic., MA-Fungi, TLXM and the private collection of D. Wrigley de Basanta (dwb). All microscopical measurements were made with material directly mounted in Hoyer's medium. For descriptive data and micrographs we used a microscope with Differential Interference-Contrast. For all SEM-pictures the critical-point technique was employed. Color notations in parentheses are from the ISCC-NBS Color-Name Charts Illustrated with Centroid Colors (Anonymous, 1976).

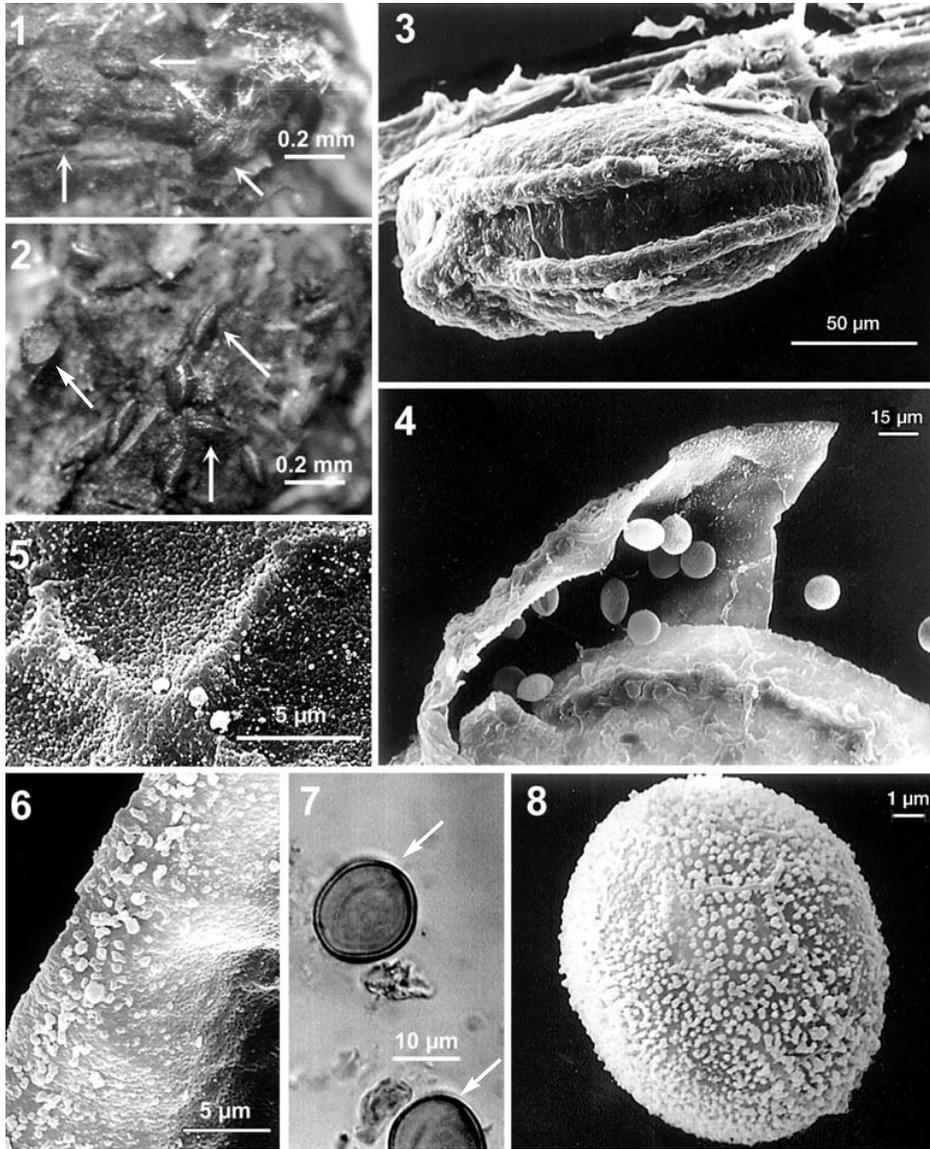
RESULTS

Licea succulenticola Mosquera, Lado, Estrada-Torres & Beltrán-Tej., **sp. nov.** (figs. 1-10)

Speciei Licea biforis Morgan proxima, differens vero sporophoris minoribus –sporocarpicis 40-110 μm diam., plasmodiocarpicis autem 40-100 × 55-170 μm– et sporis maioribus (11-14.5 × 12-17 μm) atque pariete quoad partem incrassatis, sed tenuioribus et pallidioribus quoad aliam partem.

Holotypus. SPAIN. CANARY ISLANDS: Tenerife, Barranco de Orchilla, San Miguel, 28RCS4203, alt. 100 m, on moist chamber of decaying *Euphorbia canariensis* pith skeleton, 27-III-1998, J. Mosquera, in TFC Mic. 8578 (isotypus in MA-Fungi 47348).

Sporophores sporocarpic to plasmodiocarpic, dispersed or grouped, sessile, strong yellowish brown (74. s. y Br) to deep yellow-



Figs. 1-8.—*Licea succulenticola*. 1-2, Sporophores (TFC-Mic 8225), the lighter longitudinal area is visible (arrows), on the left side of fig. 2 an open sporophore is visible; 3, Closed sporophore (SEM) (MA-Fungi 47346), longitudinal area free of refuse matter can be clearly seen. Inner membrane holds together the two halves of the peridium; 4, Dehiscent sporophore (SEM) (MA-Fungi 47347), dehiscence happens along the inner membrane which remains as torn fragments. On the inner surface of the top membrane verrucae can be seen; 5, Inner view of peridium (SEM) (MA-Fungi 47346), depressions formed by impression of the spores are visible; 6, Verrucae of the inner edge of the membrane (SEM) (MA-Fungi 47346); 7, Spores seen by LM (TFC-Mic 8225); 8, Spore (SEM) (MA-Fungi 47347), densely and minutely warted.

ish brown (75. deep y Br). Sporocarps subglobose, (40)65-170 μm diam. Plasmodiocarps elipsoid, (37)47-150(200) \times (55)75-260(400) μm , somewhat laterally compressed. Hypothallus inconspicuous. Peridium double, without peridial platelets, light yellow (86. l. Y) by LM. Inner part persistent, membranous, thin. Outer part gelatinous when moist, composed of granular and occasionally some crystalline material, lacking in an upper longitudinal, lighter area which is occasionally forked; inner peridial surface densely punctate, with dispersed verrucae on the inner side of the lighter longitudinal area; by LM granules can be noticed and are seen clearly by SEM. Columella absent. Capillitium absent. Spores free, strong yellowish brown (74. s. y Br) in mass, brilliant orange-yellow (67. brill. OY) by LM, subglobose to irregular, 11-14.5 \times 12-16(17) μm diam., minutely and densely punctate (mainly appreciable with immersion oil and SEM; fig. 8); spore wall thick on the 1/2-2/3 of the surface (0.8-1.2 μm), gradually thinner and lighter towards one side (less than 0.8 μm). Protoplasmodium colourless.

The name derives from Latin: *succulentus* (fleshy) and *cola* (dweller), referring to the characteristic ecology of this species.

Habitat. On decaying succulent plants such as *Agave americana* and *A. atrovirens* leaves, *Aeonium* sp. leaves and pith skeletons of *Austrocylindropuntia exaltata*, *Euphorbia canariensis*, *Myrtillocactus geometrizans*, *Opuntia ficus-indica*, *O. tomentosa* and *Stenocereus* sp., and bark of *Yucca filifera*, *Opuntia streptacantha*, and *Nolina laxiflora*.

Distribution. At the moment known from Tenerife (Canary Islands, Spain), the States of Hidalgo, Morelos, Puebla and Tlaxcala (Mexico) and New Jersey (USA). Probably occurring in other regions of the world where succulent plants are present.

Specimens examined

SPAIN. CANARY ISLANDS: Tenerife, Anaga Peninsula, Jardina, 28RCS7455, alt. 850 m, in moist chamber of decaying *Agave americana* leaves, 21-XII-1995, *J. Mosquera*, TFC Mic. 7319 (MA-Fungi 47346), 7408. Lomo Bermejo, Anaga, 28RCS8757, alt. 200 m, on decaying

Aeonium sp. leaves, 2-I-1996, *J. Mosquera*, TFC Mic. 7410. Tacoronte, 28RCS6152, alt. 200 m, in moist chamber of decaying *Opuntia ficus-indica* pith skeleton, 23-I-1998, *J. Mosquera*, TFC Mic. 8224 (MA-Fungi 47352), 8225, 8226. Barranco de Orchilla, San Miguel, 28RCS4203, alt. 100 m, in moist chamber of decaying *Euphorbia canariensis* pith skeleton, 23-I-1998, *J. Mosquera* & *E. Beltrán Tejera*, TFC Mic. 8390 (MA-Fungi 47347); ibidem, 24-III-1998, 8406, 8556, 8557; ibidem, 27-III-1998, TFC Mic. 8578 (Holotypus, isotypus in MA-Fungi 47348), 8581 (MA-Fungi 47349). Valle de Guerra, El Boquerón, 28RCS6453, alt. 525 m, in moist chamber of decaying *Opuntia ficus-indica* pith skeleton, 23-III-1998, *J. Mosquera* & *E. Beltrán Tejera*, TFC Mic. 8609, 8623. La Laguna, Avenida de la Trinidad, 28RCS7151, alt. 500 m, in moist chamber of decaying *Austrocylindropuntia exaltata*, 17-IV-1998, *J. Mosquera*, TFC Mic. 8672 (MA-Fungi 47351).

MEXICO. HIDALGO: Epazoyucan, Xochihuacan, 19°59'12" N, 98°42'15" W, alt. 2400 m, on *Agave* sp. leaves and skeleton, 12-X-1999, *A. Estrada-Torres*, *C. Lado*, *J.M. Ramírez-Ortega* & *M. Díaz-Ramírez* 5606, Lado 11026 (TLXM). Mezquititlán, Paso de León, km 53.5 road Pachuca-Tampico, 20°25'15" N, 98°41'12" W, in moist chamber of decaying pith skeleton of *Myrtillocactus geometrizans*, 24-XI-1998, *J.A. García-Cortés* 772-778, 781-782 (TLXM). Mezquititlán, km 69 road Pachuca-Tampico, 20°29'46" N, 98°40'04" W, in moist chamber of decaying pith skeleton of *Myrtillocactus geometrizans*, 24-XI-1998, *J.A. García-Cortés* 779-780 (TLXM); ibidem, in moist chamber of decaying pith skeleton of *Opuntia* sp., 24-XI-1998, *J.M. Ramírez-Ortega* 36-38 (TLXM). La Paloma, 19°59'59" N, 98°42'30" W, alt. 2363 m, in moist chamber of *Opuntia streptacantha* bark, 15-III-2002, dwb 2169. MORELOS: Tlaquiltenango, Reserva de la Sierra de Huautla, in moist chamber of decaying *Stenocereus* sp. pith skeleton, 12-V-1998, *A. Estrada-Torres*, *C. Lado* & *M. Rodríguez-Palma*, Lado 9138, 9140 (MA-Fungi 47350). PUEBLA: Emilio Portes Gil, 19°17'40" N, 97°30'21" W, alt. 2430 m, in moist chamber of *Yucca filifera* bark, 3-II-2001, dwb 1967. Emilio Portes Gil, 19°16'53" N, 97°29'27" W, alt. 2520 m, in moist chamber of *Nolina laxiflora* bark, 13-X-2001, dwb 2084. TLAXCALA: Ixtacuixtla, km 10.5 road San Martín Texmelucan-Tlaxcala, 19°19'42" N, 98°22'30" W, alt. 2400 m, on *Opuntia tomentosa* debris, 10-XI-1998, *A. Estrada-Torres* 5105 (TLXM). Tlaxco, El Rosario, 19°41'192 N, 98°14'002 W, alt. 2550 m, on *Agave atrovirens* leaves, 17-XI-1998, *A. Estrada-Torres* 5204 (TLXM).

USA. NEW JERSEY: On *Opuntia* leaves, 1-II-1963 and 5-IV-1963, *Ruth McVaugh Allen* H 528 (BPI 826021).

Agar culture specimens: MA-Fungi 46913, 46914 (spores obtained from TFC Mic. 8390).

Licea biforis Morgan

USA. OHIO: Preston, 1893, *Rex* 163, A.P.M. (BPI 826016, Lectotype).

CANADA. ONTARIO: London, 1893, *J. Dearness*, det. A.P.M. (BPI 826043, Syntype).

Licea pumila G.W. Martin & R. M. Allen

USA. NEW JERSEY: Riverton, on decayed inner bark of *Populus*, 24 July 1965, Ruth M. Allen C-1916 (Holotype in BPI 826421).

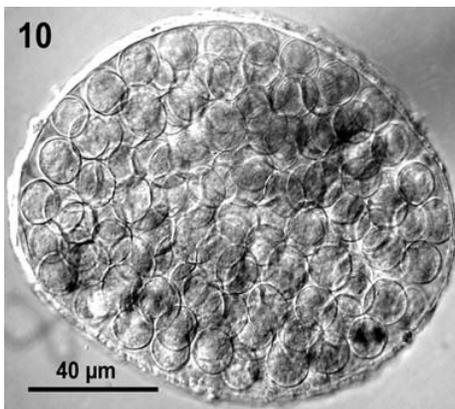
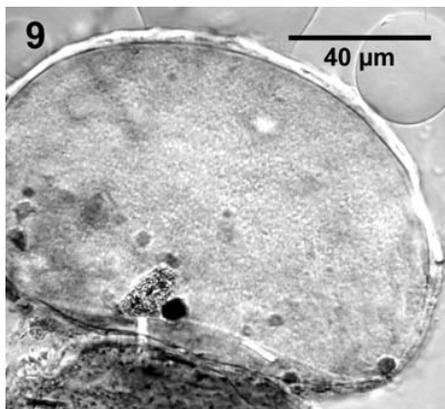
Culture. Five oatmeal-agar Petri dishes were sown with spores from the type material of *L. succulenticola*. Myxamoebae appeared one day after sowing the spores and a dense population could be observed the next day. Plasmodia appeared three days after sowing and were very similar to those of *L. biforis* described by McMANUS (1966), and of the protoplasmodium type. They moved as rounded or elongated masses, almost colourless, and fructified overnight, generally after migrating to the edges of the agar. They produced single sporophores slightly smaller in size than those directly collected in the field (40-60 × 55-65 µm) and uncoated with refuse matter. Spores could be seen through the colourless peridium and the typical lighter longitudinal area was difficult to observe due to lack of refuse matter and the lack of contrast against the agar. Spore characteristics were the same as in those of material directly collected in the field.

In moist chamber culture the colourless protoplasmodia gave rise to transparent sporocarps (fig. 9) with the thinner area of dehiscence already visible between the edges

of the slightly opened halves of the peridium, the whole structure reminiscent of a tiny hyaline bivalve (figs. 1-3). Spores were visible forming inside (fig. 10) until the sporocarp darkened with age and the lighter longitudinal area became less obvious. On drying, many of the sporocarps remained closed, but those that opened appeared like tiny circles of golden yellow spores, with the halves of the peridium bent back against the substrate (fig. 2). The incubation period on bark was from 26 to 33 days.

DISCUSSION

Licea succulenticola is similar in sporotheca form and spore colour to *L. pumila* G.W. Martin & R.M. Allen. We examined the type material of *L. pumila* and found several differences between it and *L. succulenticola*. The spores of *L. pumila* are very pale yellow, with a border that is almost hyaline, there is a clear protoplast visible in the centre of each spore, and the spore walls have no thinner area. The spores of *L. succulenticola* are uniformly orange-yellow or golden-yellow throughout, and show a tendency to collapse around a thinner area of the wall. Some of the spores of *L. pumila* are round



Figs. 9-10.—*Licea succulenticola* (dwb 2084). 9, Sporophore, early development before the spores are formed; 10, Sporophore, later stage showing developing spores inside.

(11-12.5 μm) and some are oval (10 \times 12.5 μm) while those of *L. succulenticola* are larger [11-14.5 \times 12-16(17) μm diam.]. When viewed by LM, there are obvious darker yellow edges to the two halves of the peridium of *L. succulenticola*, and torn hyaline fragments of the inner membrane that joined the halves before dehiscence protrude in places beyond the edge (fig. 4). The borders of *L. pumila* are the same colour as the rest of the peridium.

Licea succulenticola also has similarities to *L. tenera* E. Jahn and *L. punctiformis* G.W. Martin, but both lack the lighter longitudinal area of dehiscence. In addition, *L. tenera* differs in its smooth spores (minutely and densely punctate in *L. succulenticola*). The size of the sporocarps of *L. punctiformis* is similar to that of the species we describe but the spores are smaller (8-10 μm diam) and with rather sparsely distributed large warts (MARTIN & ALEXOPOULOS, 1969).

Licea succulenticola is very similar to *L. biforis* Morgan in having a somewhat compressed sporotheca with a paler longitudinal area free of refuse material. The differences however, distinguishing *L. succulenticola* from *L. biforis* are the spore size, spore color and wall thickness of the spores. *Licea* is a morphologically simple genus that presents few taxonomically helpful morphological characters. Nevertheless, shape, size and colour of spores are considered to be valuable taxonomic characters in the Myxomycetes (MARTIN & ALEXOPOULOS, 1969). In order to check the validity of the different characters between both species, we studied the lectotype of *L. biforis* and a syntype kept at the BPI, and found clear and constant differences in the spore characteristics. *Licea biforis* type had brilliant yellow (83. brill. Y) spores in mass and they were strong yellowish brown (74. s. y Br) in *L. succulenticola*. By LM *L. biforis* spores were light yellow (86. l. Y), and orange-yellow (67. brill. OY) in *L. succulenticola*. The more variable shape of the spores of *L. biforis*, their smaller size (7.2-8 \times 8.8-11.2 μm vs. 11-14.5 \times 12-17 μm), and a homogeneously thin spore wall also differentiate the two, whereas the spores have thick

walls which are thinner and lighter towards one side in *L. succulenticola* (fig. 7). GILBERT (1997) reported that in TEM longitudinal sections, *L. biforis* lacks a thinner portion on the spore wall, it being of equal thickness.

In addition to the spore differences, *L. succulenticola* differs from *L. biforis* in its smaller sporotheca (47-150 \times 75-260 μm vs. 100-300 \times 200-1500 μm), in not having as long a fusiform shape, and in never being long plasmodiocarpous (MARTIN & ALEXOPOULOS, 1969; MCMANNUS & GRONEN, 1966). It has a smaller length to width ratio. Both species share the possession of granules all over the inner side of the peridium (GILBERT, 1997) and the possession of warts on the inner surface of the lighter longitudinal area (NANNENGA-BREMEKAMP, 1965) (figs. 4-6). Granules also occur in *L. retiformis* Nawawi as shown by GILBERT (1987), so are not an exclusive character.

We have also found material of *L. succulenticola* within the BPI myxomycete collection (BPI 826021). It was collected in New Jersey (USA) from *Opuntia* leaves and labelled as "*Licea ?biforis* Morg.". Inside the box a quote stated "See letter of R.M. Allen to Farr 5 IV 1963.". We tried to find that letter within the BPI archives but we were unsuccessful. It seems likely that R.M. Allen noticed that material from *Opuntia* was different to some extent from *L. biforis* and asked for help from M.L. Farr. We examined Allen's specimen and it concurred with the characteristics of the new species described here.

In order to check that the differing characters between *L. biforis* and *L. succulenticola* are due to genetic differences and not to different environmental growing conditions, we cultured *L. succulenticola* from spore to spore in controlled conditions. In our cultures, laboratory conditions present from germination throughout fructification were: pH = 7, temperature of about 20 $^{\circ}\text{C}$ and no chemical compounds or microflora from decaying *Opuntia* cladodia present. These conditions are very different from those in the decaying cladodia: alkaline pH, temperatures higher than 20 $^{\circ}\text{C}$ normally, presence of complex chemical compounds from the plant and of a complex microflora composed of different species of

bacteria and yeasts (MOSQUERA & *al.*, 2000a). Even so, the spores obtained from sporophores in culture presented the same characteristics as those from specimens collected in the field confirming their characters as constant.

Licea biforis has been cultured in laboratory by other authors and spore characteristics were typical of that species (MCMANNUS, 1966; MCMANNUS & GRONEN, 1966; WOLLMAN & ALEXOPOULOS, 1964, 1967). WOLLMAN & ALEXOPOULOS (1967) reported that sporophores obtained from spore to spore on agar produce spores that were pale yellow under oil immersion, and were about 10.5 μ m diam. Thus it seems that *L. succulenticola* has morphological differences to *L. biforis* that are stable, and reflect genomic variation and not environmental responses.

Ecology. Myxomycetes from the genus *Licea* occur frequently on bark of living trees (KELLER & BROOKS, 1977). They have protoplasmodia, the same as *Macbrideola* and *Echinostelium*, other genera that fructify abundantly in this habitat. The possession of this type of plasmodium seems to be an adaptation to a habitat with a high risk of desiccation (LADO & *al.*, 1999). Succulent plants usually grow in arid lands, where the environmental conditions, in which succulenticolous myxomycete plasmodia develop, can be considered to be similar to those on the bark of some living trees. Thus the appearance of *Licea* species on this substratum is not surprising. BLACKWELL & GILBERTSON (1980a) already cited *L. parasitica* (Zukal) G.W. Martin and *L. pedicellata* (H.C. Gilbert) H.C. Gilbert on *Opuntia* spp. from the Arizona Desert, and we have found *Licea kleistobolus* G.W. Martin on *Stenocereus* pith skeletons. *Licea succulenticola* appeared abundantly in moist chamber from material collected from large arid areas of Mexico and the Canary Islands.

The abiotic parameters of decaying succulent plants, however, are different from those of the bark of living trees. The pH of the former substrata was usually alkaline in the moist chamber cultures from which we have ob-

tained *L. succulenticola* fructifications: *Stenocereus*, pH 10; *Opuntia ficus-indica*, pH 8-10; *Euphorbia canariensis*, pH 8-9.5; *Agave*, pH 7.5-10.3. BLACKWELL & GILBERTSON (1984) reported a pH of 8.7-10.4 for dead saguaro tissue (*Carnegiea gigantea*) although *Yucca filifera* bark pH 7.0, *Nolina laxiflora* bark pH 6.8, and *Opuntia streptacantha* bark pH 7.2 were more neutral. Almost all these values are higher than those obtained for bark of living trees (HÄRKÖNEN, 1977, 1978; UKKOLA, 1998). Other important differences between both substrata include the porosity of the material, the water-holding capacity, temperature and nutrient concentrations. In addition, the microbial biota is very different (FOGLEMAN & FOSTER, 1989) and there may be competition for food among myxocells and different invertebrates (MOSQUERA & *al.*, 2000a). *Licea succulenticola* seems to be specialised for this particular habitat, as are the other succulenticolous species *D. eremophilum* Blackwell & Gilbertson (BLACKWELL & GILBERTSON, 1980b), *Cribraria zonatispora* Lado, Mosquera & Beltrán-Tej. (LADO & *al.*, 1999), *Trichia agaves* (G. Moreno, Lizárraga & Illana) Mosquera, Lado, Estrada & Beltrán-Tej. (= *T. perichaenoides* Mosquera, Lado, Estrada & Beltrán-Tej.) (LADO, 2001; MOSQUERA & *al.*, 2000b) and *Didymium subreticulosporum* Oltra, G. Moreno & Illana (MORENO & *al.*, 1996).

We have found *L. biforis* in the same environment as *L. succulenticola* (decaying *Agave*), each species showing its different characters. After four years studying the succulenticolous habitat, however, we have only found one sample of *L. biforis* on decaying succulent plants, in contrast to the numerous and abundant samples of *L. succulenticola*, showing that this new species is better adapted to the succulenticolous habitat. We have collected it on nine different decaying succulent plants and the bark of three other species in xeric habitats. It has also been found on two continents, off the coast of Africa (the Canary Islands) and in America (Mexico and USA), although it probably occurs in other regions of the world where succulent plants are present.

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